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| (51) International Patent Classification ⁶ : A61K 38/00, 31/725, 31/195 | A1 | (11) International Publication Number: WO 00/06184 (43) International Publication Date: 10 February 2000 (10.02.00) |
| (21) International Application Number: PCT/US99/16957 (22) International Filing Date: 27 July 1999 (27.07.99) (30) Priority Data: 60/094,267 27 July 1998 (27.07.98) US 60/104,466 16 October 1998 (16.10.98) US (71) Applicant (for all designated States except US): EMISPHERE TECHNOLOGIES, INC. [US/US]; 765 Old Saw Mill River Road, Tarrytown, NY 10591 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): MILSTEIN, Sam, J. [US/US]; 105 Willow Avenue, Larchmont, NY 10538 (US). SMART, John, E. [US/US]; 22 Holly Hill Lane, Katonah, NY 10536 (US). SARUBBI, Donald, J. [US/US]; 64 Gail Court, Carmel, NY 10512 (US). CAROZZA, Monica [US/US]; 14 Roundabend Road, Tarrytown, NY 10591 (US). FLANDERS, Elizabeth [US/US]; 421 North Street, Ridgefield, CT 06877 (US). O'TOOLE, Doris [US/US]; 33 Lakeview Road, Carmel, NY 10512 (US). LEONE-BAY, Andrea [US/US]; 20 Woodland Way, Ridgefield, CT 06877 (US). GSCHNEIDNER, David [US/US]; 44 Cerretta Street, Stamford, CT 06907 (US). | | (74) Agents: LESSLER, Jay, P. et al.; Darby & Darby P.C., 805 Third Avenue, New York, NY 10022-7513 (US). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> |
| (54) Title: PULMONARY DELIVERY OF ACTIVE AGENTS (57) Abstract Methods of administration of active agents via the pulmonary route are provided. | | |

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PULMONARY DELIVERY OF ACTIVE AGENTS

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FIELD OF THE INVENTION

The present invention relates to pulmonary delivery of active agents. Acylated or sulfonated amino acids are used as carriers to facilitate pulmonary delivery of active agents to a target.

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BACKGROUND OF THE INVENTION

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Conventional means for delivering active agents are often severely limited by biological, chemical, and physical barriers. Typically, these barriers are imposed by the environment through which delivery occurs, the environment of the target for delivery, or the target itself. Biologically or chemically active agents are particularly vulnerable to such barriers.

30

For example, in the delivery to animals of biologically active or chemically active pharmacological and therapeutic agents, barriers are imposed by the body. Examples of physical barriers are the skin and various organ membranes that must be traversed before reaching a target. Chemical barriers include, but are not limited to, pH variations, lipid bi-layers, and degrading enzymes.

Pulmonary delivery to the circulatory system for many biologically active agents could be the route of choice for administration to animals because delivery to the blood is much

5 more rapid than with other routes of delivery. In addition, delivery to the lung itself may be desired, e.g., for treatment of ailments of the pulmonary system. However, pulmonary delivery may not be practical because of physical barriers such as lipid bi-layers, and membranes that are relatively impermeable to certain biologically active agents, but which must be traversed before an agent can reach the circulatory system. In other cases, pulmonary delivery may be achieved,
10 but not efficiently enough for practical purposes.

There is presently a need for simple, inexpensive pulmonary delivery systems which are easily prepared and which can deliver a broad range of active agents in an efficient manner.

BRIEF DESCRIPTION OF THE DRAWINGS

15 Figure 1 is a graphical illustration of plasma insulin-time profiles following lung spray-IT instillation of insulin alone 0.05 mg/kg (0.13U/kg) (◆) and combined with Carrier B 5 mg/kg (■). Bars represent \pm SD n=5.

Figure 2 is a graphical illustration of plasma glucose-time profiles following lung spray-IT instillation of insulin alone 0.05 mg/kg (0.13 U/kg) (◆) and combined with Carrier B
20 5 mg/kg (■). Bars represent \pm SD n=5.

Figure 3 is a graphical illustration of plasma glucose-time profiles following lung spray-IT instillation of insulin alone. 0.05 mg/kg (0.13 U/kg) (◆) and combined with Carrier B 16 mg/kg (■). Bars represent \pm SD n=3.

Figure 4 is a graphical illustration of plasma glucose-time profiles following lung
25 spray-IT instillation of insulin alone 0.01 mg/kg (0.026U/kg) (◆) and combined with Carrier B 16 mg/kg, (■). Bars represent \pm SD n=4.

Figure 5 is a graphical illustration of insulin average plasma concentration over time following lung spray-IT instillation of 0.005 mg/kg (0.013 U/kg) (*), 0.05 mg/kg (0.13 U/kg) (◆), 0.1 mg/kg (0.26 U/kg) (^), 0.5 mg/kg (1.3 U/kg) (■), and 1 mg/kg (2.6 U/kg) (●) porcine
30 insulin to rats. Bars represent \pm SD.

Figure 6 is a graphical illustration of plasma glucose-time profiles following spray-IT instillation of 0.01 mg/kg (0.026 U/kg) (*), 0.05 mg/kg (0.13 U/kg) (x), 0.1 mg/kg (0.26 U/kg) (◆), 0.5 mg/kg (1.3 U/kg) (■), and 1 mg/kg (2.6 U/kg) (^) porcine insulin to rats. Bars represent \pm SD.

35 Figure 7 is a graphical illustration of insulin plasma concentration over time of 0.05 mg/kg insulin with and without Carrier B 16 mg/kg..

5 Figure 8 is a graphical illustration of insulin plasma concentration over time with varying dose of insulin.

 Figure 9 is a graphical illustration of insulin plasma concentration over time of 0.05 mg/kg insulin with and without Carrier B 5 mg/kg.

 Figure 10 is a graphical illustration of insulin plasma concentration over time of
10 0.01 mg/kg insulin with and without Carrier B 16 mg/kg.

 Figure 11 is a graphical illustration of percent change in blood glucose following pulmonary delivery of 0.1 mg insulin with 7.5 mg/kg Carrier B sodium salt (■), 0.1 mg/kg insulin alone (●), and 7.5 mg/kg Carrier B sodium salt alone (▲).

 Figure 12 is a graphical illustration of percent change in blood glucose following
15 pulmonary delivery of 0.5 mg insulin with 7.5 mg/kg Carrier B sodium salt (■), 0.5 mg/kg insulin alone (●), and 7.5 mg/kg Carrier B sodium salt alone (▲).

 Figure 13 is a graphical illustration of serum insulin levels over time of 0.03 mg/kg insulin with 16 mg/kg Carrier B (●), Carrier C (▲), and Carrier D (▼), and insulin alone (■). Bars represent \pm SD.

20 SUMMARY OF THE INVENTION

 The present invention provides methods for administering an active agent to an animal in need of the agent by the pulmonary route. This method comprises administering via the pulmonary route, a composition comprising (a) an active agent and (b)(i) an acylated amino acid, (ii)
25 a sulfonated amino acid, or (iii) a combination of (i) and (ii). Administration of the compositions of the present invention provide improved pulmonary delivery and greater bioavailability of the active agent than administration of the active agent alone. As a result, lesser amounts of the active agent may be administered to obtain a desired result when contained in the composition of the present invention than when administered alone.

30 DETAILED DESCRIPTION OF THE INVENTION

 Compositions useful in the present invention include an active agent and a carrier. These compositions may be used to deliver various active agents through various biological, chemical, and physical barriers and are particularly suited for delivering active agents which are
35 subject to environmental degradation. The methods of the subject invention are particularly useful for delivering or administering biologically, pharmacologically, or chemically active agents to any

5 animal, including, but not limited to, birds, such as chickens, and mammals, such as cows, pigs, dogs, cats, primates, and particularly humans.

Pulmonary coadministration of a carrier with an active agent, such as, for example insulin as described herein results in an increased bioavailability of the active agent compared to administration of the active agent alone.

Active Agents

Active agents suitable for use in the present invention include biologically or chemically active agents.

Biologically or chemically active agents include, but are not limited to, pesticides, pharmacological agents and therapeutic agents. For example, biologically or chemically active agents suitable for use in the present invention include, but are not limited to, proteins; polypeptides; peptides; hormones, and particularly hormones which by themselves do not or only a fraction of the administered dose passes through the alveoli of the lung and/or are susceptible to chemical cleavage by acids and enzymes in the lung; polysaccharides, and particularly mixtures of muco-polysaccharides; carbohydrates; lipids; other organic compounds; or any combination thereof.

Further examples include, but are not limited to, the following, including synthetic, natural or recombinant sources thereof; growth hormones, including human growth hormones (hGH), recombinant human growth hormones (rhGH), bovine growth hormones, and porcine growth hormones; growth hormone-releasing hormones; interferons, including α , β and γ ; interleukin-1; interleukin II; insulin, including porcine, bovine, human and human recombinant, optionally having counter ions including sodium, zinc, calcium, and ammonium; insulin-like growth factor (IGF), including IGF-1; heparin, including unfractionated heparin, heparinoids, dermatans, chondroitins, low molecular weight heparin, very low molecular weight heparin and ultra low molecular weight heparin; calcitonin, including salmon, eel and human; erythropoietin; atrial natureic factor; antigens; monoclonal antibodies; somatostatin; protease inhibitors; adrenocorticotropin, gonadotropin releasing hormone; oxytocin; leutinizing-hormone-releasing-hormone; follicle stimulating hormone; glucocerebrosidase; thrombopoietin; filgrastim; prostaglandins; cyclosporin; vasopressin; cromolyn sodium (sodium or disodium chromoglycate); vancomycin; desferrioxamine (DFO); parathyroid hormone (PTH), including its fragments; antimicrobials, including anti-fungal agents; analogs, fragments, mimetics or polyethylene glycol (PEG)-modified derivatives of these compounds; or any combination thereof.

Carriers

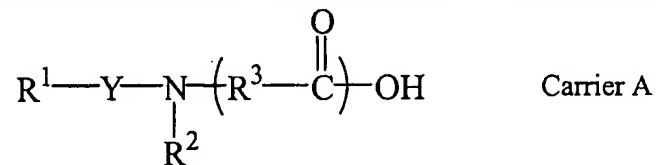
Acylated and sulfonated amino acids have been found to act as carriers for pulmonary delivery of biologically or chemically active agents. These carrier compounds or poly amino acids and peptides, may be used to deliver active agents including, but not limited to, biologically or chemically active agents, such as for example, pharmacological and therapeutic agents.

An amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring and synthetic amino acids. Poly amino acids are either peptides or two or more amino acids linked by a bond formed by other groups which can be linked, e.g. an ester, anhydride, or an anhydride linkage. Peptides are two or more amino acids joined by a peptide bond. Peptides can vary in length from dipeptides with two amino acids to polypeptides with several hundred amino acids. See Chambers Biological Dictionary, editor Peter M.B. Walker, Cambridge, England: Chambers Cambridge, 1989, page 215. Special mention is made of di-peptides, tri-peptides, tetra-peptides, and penta-peptides.

Amino acids may be used to prepare the carriers useful in the present invention. An amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring and synthetic amino acids. Many amino acids and amino acid esters are readily available from a number of commercial sources such as Aldrich Chemical Co. (Milwaukee, Wis, USA); Sigma Chemical Co. (St. Louis. Mo., USA); and Fluka Chemical Corp. (Ronkonkoma, N.Y., USA). Peptides can be homo- or hetero-peptides and can include natural amino acids, synthetic amino acids, or any combination thereof.

Modified amino acids, poly amino acids, or peptides are either acylated or sulfonated and include amino acid amides and sulfonamides.

Special mention is made of acylated or sulfonated amino acids having the formula



wherein R¹ is C₁-C₇ alkyl, C₃-C₁₀ cycloalkyl, cycloalkenyl, aryl, thienyl, phenyl, naphthyl, pyrrolo, or pyridyl;

R¹ is optionally substituted with one or more C₁-C₇ alkyl, C₂-C₇ alkenyl, C₂-C₇ alkynyl, C₆-C₁₀ cycloalkyl, phenyl, phenoxy, F, Cl, Br, -OH, -SO₂, -SO₃H, -NO₂, -SH, -PO₃H, oxazolo, isoxazolo, alkoxy having the formula -OR⁶, -COOR⁷, -N(R⁵)₂, -N⁺(R⁵)₃X⁻, or any combination thereof;

5 Y is $\text{—}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{—}$ or $\text{—SO}_2\text{—}$;
 X is halogen, hydroxide, sulfate, tetrafluoroborate, or phosphate;
 R^2 is hydrogen, $\text{C}_1\text{—C}_4$ alkyl, $\text{C}_2\text{—C}_4$ alkenyl, or $\text{—(CH}_2\text{)}_n\text{—COOH}$, where n is 1 to 10;
 R^3 is $\text{C}_1\text{—C}_{24}$ alkyl, $\text{C}_2\text{—C}_{24}$ alkenyl, $\text{C}_2\text{—C}_{24}$ alkyne, $\text{C}_3\text{—C}_{10}$ cycloalkyl, $\text{C}_3\text{—C}_{10}$
 cycloalkenyl, phenyl, naphthyl, $(\text{C}_1\text{—C}_{10}$ alkyl) phenyl, $(\text{C}_2\text{—C}_{10}$ alkenyl) phenyl, $(\text{C}_1\text{—C}_{10}$ alkyl)
 10 naphthyl, $(\text{C}_2\text{—C}_{10}$ alkenyl) naphthyl, phenyl $(\text{C}_1\text{—C}_{10}$ alkyl), phenyl $(\text{C}_2\text{—C}_{10}$ alkenyl), naphthyl $(\text{C}_1\text{—C}_{10}$ alkyl) or naphthyl $(\text{C}_2\text{—C}_{10}$ alkenyl);

R^3 being optionally substituted with $\text{C}_1\text{—C}_4$ alkyl, $\text{C}_2\text{—C}_4$ alkenyl, $\text{C}_1\text{—C}_4$ alkoxy, —OH ,
 —SH , halogen, —NH_2 , $\text{—CO}_2\text{R}^4$, $\text{C}_3\text{—C}_{10}$ cycloalkyl, $\text{C}_3\text{—C}_{10}$ cycloalkenyl, heterocyclic having 3-10 ring
 atoms wherein the hetero atom is one or more of N, O, S or any combination thereof, aryl, $(\text{C}_1\text{—C}_{10}$
 15 alk)aryl, ar $(\text{C}_1\text{—C}_{10}$ alkyl), or any combination thereof;

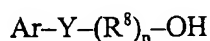
R^4 is hydrogen, $\text{C}_1\text{—C}_4$ alkyl, or $\text{C}_2\text{—C}_4$ alkenyl;

R^5 is hydrogen or $\text{C}_1\text{—C}_{10}$ alkyl;

R^6 is $\text{C}_1\text{—C}_{10}$ alkyl, alkenyl, alkynyl, aryl or cycloalkyl; and

R^7 is hydrogen, $\text{C}_1\text{—C}_{10}$ alkyl, alkenyl, alkynyl, aryl or cycloalkyl.

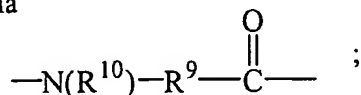
20 Special mention is also made of acylated or sulfonated amino acids having the
 formula



Carrier A'

wherein Ar is a substituted or unsubstituted phenyl or naphthyl, preferably Ar is substituted or
 unsubstituted 2-OH-phenyl;

25 Y is $\text{—}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{—}$ or $\text{—SO}_2\text{—}$;
 R^8 has the formula



R^9 is C_1 to C_{24} alkyl, C_1 to C_{24} alkenyl, phenyl, naphthyl, $(\text{C}_1$ to C_{10} alkyl) phenyl,
 30 $(\text{C}_1\text{—C}_{10}$ alkenyl) phenyl, $(\text{C}_1$ to C_{10} alkyl) naphthyl, $(\text{C}_1$ to C_{10} alkenyl) naphthyl, phenyl $(\text{C}_1$ to C_{10} alkyl), phenyl $(\text{C}_1$ to C_{10} alkenyl), naphthyl $(\text{C}_1$ to C_{10} alkyl) and naphthyl $(\text{C}_1$ to C_{10} alkenyl);

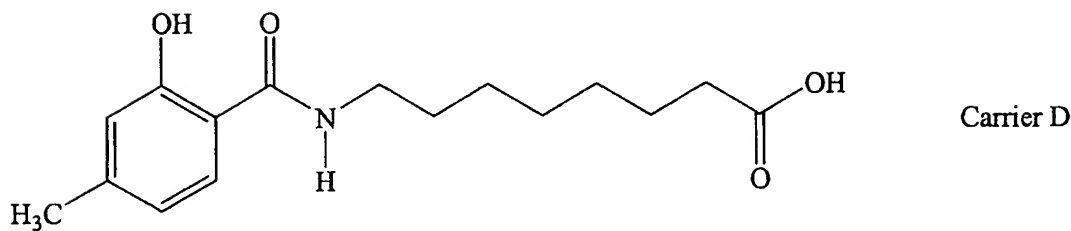
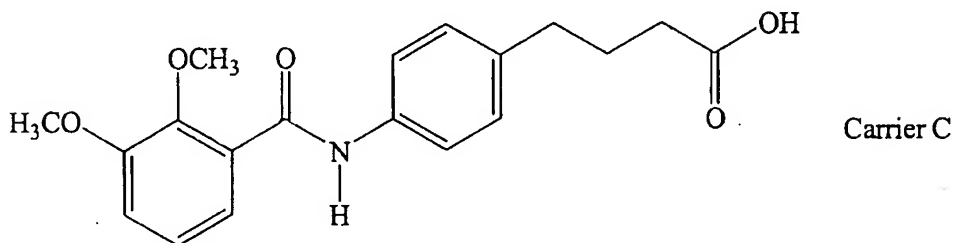
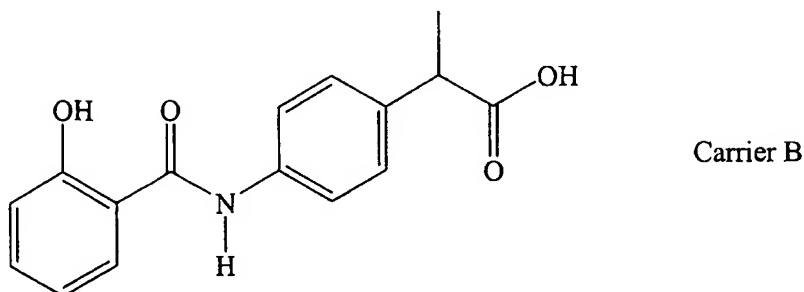
R^9 is optionally substituted with C_1 to C_4 , alkyl, C_1 to C_4 alkenyl, C_1 to C_4 alkoxy,
 —OH , —SH , $\text{—CO}_2\text{R}^{11}$, cycloalkyl, cycloalkenyl, heterocyclic alkyl, alkaryl, heteroaryl, heteroalkaryl,
 or any combination thereof;

35 R^9 is optionally interrupted by oxygen, nitrogen, sulfur or any combination thereof;

R^{10} is hydrogen, C_1 to C_4 alkyl or C_1 to C_4 alkenyl; and

R¹¹ is hydrogen, C₁ to C₄ alkyl or C₁ to C₄ alkenyl.

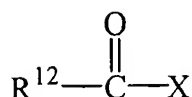
Special mention is also made of the following compounds and their salts, including, but not limited to, sodium salts thereof.



Some preferred delivery agents include, but are not limited to, those described in U.S. Patent Nos. 4,925,673; 5,451,410; 5,541,155; 5,629,020; 5,643,957; 5,650,386; 5,709,861; 5,714,167; 5,766,633; 5,773,647; 5,792,451; and 5,863,944 and PCT Publication Nos. WO96/12474; WO97/10197; WO97/36480; and WO98/50341.

Acylated amino acids may be prepared by reacting single amino acids, mixtures of two or more amino acids, or amino acid esters with an amine modifying agent which reacts with free amino moieties present in the amino acids to form amides.

Suitable, but not limiting, examples of acylating agents useful in preparing an acylated amino acids include the acid chloride acylating agents having the formula



wherein R¹² is an appropriate group for the modified amino acid being prepared, such as, but not limited to, alkyl, alkenyl, cycloalkyl, or aromatic, and particularly methyl, ethyl, cyclohexyl, cyclophenyl, phenyl, or benzyl; and X is a leaving group. Typical leaving groups include, but are not limited to, halogens such as chlorine, bromine and iodine.

10 Examples of the acylating agents include, but are not limited to, acyl halides including, but not limited to, acetyl chloride, propyl chloride, cyclohexanoyl chloride, cyclopentanoyl chloride, and cycloheptanoyl chloride, benzoyl chloride, hippuryl chloride and the like; and anhydrides, such as acetic anhydride, propyl anhydride, cyclohexanoic anhydride, benzoic anhydride, hippuric anhydride and the like. Preferred acylating agents include benzoyl chloride, hippuryl chloride, acetyl chloride, cyclohexanoyl chloride, cyclopentanoyl chloride, and cycloheptanoyl chloride.

20 The amine groups can also be modified by the reaction of a carboxylic acid with coupling agents such as the carbodiimide derivatives of amino acids, particularly hydrophilic amino acids such as phenylalanine, tryptophan, and tyrosine. Further examples include dicyclohexylcarbodiimide and the like.

25 If the amino acid is multifunctional, i.e., has more than one --OH, -NH, or --SH group, then it may optionally be acylated at one or more functional groups to form, for example, an ester, amide, or thioester linkage.

30 For example, in the preparation of many acylated amino acids, the amino acids are dissolved in an aqueous alkaline solution of a metal hydroxide, e.g., sodium or potassium hydroxide and the acylating agent added. The reaction time can range from about 1 hour to about 4 hours, preferably about 2 to about 2.5 hours. The temperature of the mixture is maintained at a temperature generally ranging between about 5° C and about 70°C., preferably between about 10° C. and about 50°C. The amount of alkali employed per equivalent of NH₂ groups in the amino acids generally ranges between about 1.25 moles and about 3 moles, and is preferably between about 1.5 moles and about 2.25 moles per equivalent of NH₂. The pH of the reaction solution generally ranges between about pH 8 and about pH 13, and is preferably between about pH 10 and about pH 12. The amount of amino modifying agent employed in relation to the quantity of amino acids is based on the moles of total free NH₂ in the amino acids. In general, the amino modifying agent is

35

5 employed in an amount ranging between about 0.5 and about 2.5 mole equivalents, preferably between about 0.75 and about 1.25 equivalents, per molar equivalent of total NH₂ groups in the amino acids.

The modified amino acid formation reaction is typically quenched by adjusting the pH of the mixture with a suitable acid, e.g., concentrated hydrochloric acid, until the pH reaches
10 between about 2 and about 3. The mixture separates on standing at room temperature to form a transparent upper layer and a white or off-white precipitate. The upper layer is discarded, and modified amino acids are collected by filtration or decantation. The crude modified amino acids are then mixed with water. Insoluble materials are removed by filtration, and the filtrate is dried in vacuo. The yield of modified amino acids generally ranges between about 30 and about 60%,
15 and usually about 45%. The present invention also contemplates amino acids which have been modified by multiple acylation, e.g., diacylation, triacylation, etc.

If amino acid esters or amides are the starting materials, they are dissolved in a suitable organic solvent such as dimethylformamide or pyridine, and are reacted with the amino
20 modifying agent at a temperature ranging between about 5°C. and about 70° C, preferably about 250°C, for a period ranging between about 7 and about 24 hours. The amount of amino modifying agents used relative to the amino acid esters are the same as described above for amino acids.

Thereafter, the reaction solvent is removed under negative pressure, and optionally, the ester or amide functionality can be removed by hydrolyzing the modified amino acid ester with
25 a suitable alkaline solution, e.g., 1N sodium hydroxide, at a temperature ranging between about 50°C, and about 80°C., preferably about 70°C., for a period of time sufficient to hydrolyze off the ester group and form the modified amino acid having a free carboxyl group. The hydrolysis mixture is then cooled to room temperature and acidified, e.g., with an aqueous 25% hydrochloric acid solution, to a pH ranging between about 2 and about 2.5. The modified amino acid precipitates out of solution and is recovered by conventional means such as filtration or
30 decantation.

The modified amino acids may be purified by acid precipitation, recrystallization, or fractionation on solid column supports. Fractionation may be performed on a suitable solid
35 column supports such as silica gel or alumina, using solvent mixtures such as acetic acid/butanol/water as the mobile phase; reverse phase column supports using trifluoroacetic acid/acetonitrile mixtures as the mobile phase, and ion exchange chromatography using water as the mobile phase. The modified amino acids may also be purified by extraction with a lower

5 alcohol such as methanol, butanol, or isopropanol to remove impurities such as inorganic salts.

The modified amino acids generally are soluble in alkaline aqueous solution (pH \geq 9.0); partially soluble in ethanol, n-butanol and 1:1 (v/v) toluene/ethanol solution; and insoluble in neutral water. The alkali metal salts, e.g., the sodium salts of the modified amino acids, are generally soluble in water at about a pH of 6-8.

10 In poly amino acids or peptides, one or more of the amino acids may be modified acylated and/or sulfonated. Poly amino acids and peptides may include one or more acylated amino acid(s). Although linear modified poly amino acids and peptides will generally include only one acylated amino acid, other poly amino acid and peptide configurations can include more than one acylated amino acid. Poly amino acids and peptides can be polymerized with the acylated
15 amino acid(s) or can be acylated after polymerization.

Sulfonated amino acids, poly amino acids, and peptides are modified by sulfonating at least one free amine group with a sulfonating agent which reacts with at least one of the free amine groups present.

Suitable, but non-limiting, examples of sulfonating agents useful in preparing
20 sulfonated amino acids include sulfonating agents having the formula $R^{13}-SO_2-X$ wherein R^{13} is an appropriate group for the modified amino acid being prepared such as, but not limited to, alkyl, alkenyl, cycloalkyl, or aromatics and X is a leaving group as described above. One example of a sulfonating agent is benzene sulfonyl chloride.

Modified poly amino acids and peptides may include one or more sulfonated amino
25 acid(s) and/or acylated amino acids. Although linear modified poly amino acids and peptides used generally include only one sulfonated amino acid, other poly amino acid and peptide configurations can include more than one sulfonated amino acid. Poly amino acids and peptides can be polymerized with the sulfonated amino acid(s) or can be sulfonated after polymerization.

30 Delivery systems

The compositions useful herein may include one or more active agents.

In one embodiment, the compounds above or salts of these compounds or poly amino acids or peptides that include at least one of these compounds or salts may be used directly as a delivery carrier by simply mixing one or more compound or salt, poly amino acid or peptide
35 with the active agent prior to administration.

The administration mixtures are prepared by mixing an aqueous solution of the

5 carrier with an aqueous solution of the active ingredient, just prior to administration. Alternatively, the carrier and the biologically or chemically active ingredient can be admixed during the manufacturing process. The solutions may optionally contain additives such as phosphate buffer salts, citric acid, acetic acid, gelatin, and gum acacia.

10 Stabilizing additives may be incorporated into the carrier solution. With some drugs, the presence of such additives promotes the stability and dispersibility of the agent in solution.

1 The stabilizing additives may be employed at a concentration ranging between about 0.1 and 5% (w/v), preferably about 0.5% (w/v). Suitable, but non-limiting examples of stabilizing additives include gum acacia, gelatin, methyl cellulose, polyethylene glycol, carboxylic acids and salts thereof, and polylysine. The preferred stabilizing additives are gum acacia, gelatin, and methyl cellulose.

20 The amount of active agent is an amount effective to accomplish the purpose of the particular active agent for the target indication. The amount of active agent in the compositions typically is a pharmacologically, biologically, therapeutically, or chemically effective amount. However, the amount can be less than a pharmacologically, biologically, therapeutically, or chemically effective amount when the composition is used in a dosage unit form, such as a powder or a liquid, because the dosage unit form may contain a plurality of carrier/biologically or chemically active agent compositions or may contain a divided pharmacologically, biologically, therapeutically, or chemically effective amount. The total effective amount can then be administered in cumulative units containing, in total, pharmacologically, biologically, therapeutically or chemically active amounts of biologically or pharmacologically active agent.

25 The total amount of active agent, and particularly biologically or chemically active agent, to be used can be determined by those skilled in the art. However, it has surprisingly been found that with some biologically or chemically active agents, the use of the presently disclosed carriers in pulmonary delivery systems provides extremely efficient delivery. Therefore, lower amounts of biologically or chemically active agents than those used in prior dosage unit forms or delivery systems can be administered to the subject, while still achieving the same blood levels and therapeutic effects.

30 The amount of carrier in the present compositions is a delivery effective amount and can be determined for any particular carrier or biologically or chemically active agent by methods known to those skilled in the art. The effective amounts of the active agent and carrier

5 in the composition may vary over a considerable range and is dependent upon the age, weight, sex, sensitivity, medical history, and the like of the individual. One should take into account the nature of the active agent and carrier, the specific activity of the agent (units of bioactivity/mass), and its rate of absorption in the lung, all of which contribute to a determination of the therapeutically effective dose.

10 Following administration, the active agent present in the composition or dosage unit form is rapidly taken up into the circulation. The bioavailability of the active agent is readily assessed by measuring a known pharmacological activity in blood, e.g. an increase in blood clotting time caused by heparin, a decrease in circulating calcium levels caused by calcitonin, or blood glucose level variations caused by insulin. Alternatively, the circulating levels of active agent itself can be measured directly.

15 Alternately, where the target is the lung, delivery is effected automatically. The bioavailability of the active agent is assessed by measuring a known pharmacodynamic parameter of activity.

Dosage unit forms can also include any of excipients, diluents, disintegrants, lubricants, plasticizers, colorants, and dosing vehicles, including, but not limited to water, 1,2-propane diol, ethanol, olive oil, or any combination thereof.

20 The delivery compositions may also include one or more enzyme inhibitors. Such enzyme inhibitors include, but are not limited to, compounds such as actin in or epiactinonin and derivatives thereof. Derivatives of these compounds are disclosed in U.S. Patent No. 5,206,384, the disclosure of which is hereby incorporated herein by reference. Other enzyme inhibitors include, but are not limited to, aprotinin (Trasylol) and Bowman-Birk inhibitor.

25 The system is particularly advantageous for delivering chemically or biologically active agents which would otherwise be destroyed or rendered less effective by conditions encountered before the active agent reaches its target zone (i.e. the area in which the active agent of the delivery composition is to be released) and within the body of the animal to which they are administered. Particularly, the present invention is useful in pulmonary administration, such as by an inhaler, of active agents, especially those which are not ordinarily deliverable by that route or for which improved delivery is desirable. Improved delivery may be observed in a number of ways, including but not limited to, an overall increase in the amount of active agent delivered over time, an overall increase in the biological response over time, and an increased delivery or response at a particular time, such as in quicker delivery of the active agent or quicker response.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following examples illustrate the invention without limitation. All parts are given by weight unless otherwise indicated.

Example 1 - Preparation of Carriers

Preparation of 2-(4-(N-salicyloyl)aminophenyl) propionic acid (Carrier B)

A slurry of 58.6 g (0.355 mol) of 2-(4-aminophenyl)propionic acid and 500 ml of methylene chloride was treated with 90.11 ml (77.13 g, 0.710 mol) of trimethylsilyl chloride and was heated to reflux for 120 min. The reaction mixture was cooled to 0°C and treated with 184.44 ml (107.77 g, 1.065 mol) of triethylamine. After stirring for 5 minutes, this mixture was treated with a solution of 70.45 g (0.355 mol) of O-acetylsalicyloyl chloride and 150 ml of methylene chloride. The reaction mixture was warmed to 25°C and stirred for 64 hr. The volatiles were removed in vacuo. The residue was stirred in 2N aqueous sodium hydroxide for one hour and acidified with 2 M aqueous sulfuric acid. The solid was recrystallized twice from ethanol/water to give a tan solid. Isolation by filtration afforded 53.05 g of (52% yield) of 2-(4-(N-salicyloyl)aminophenyl)propionic acid.

Properties. Solubility: 200 mg/ml: 200 mg + 350 µl 2N NaOH + 650 µl H₂O - pH - 7.67. Anal. calculated for - C:67.36, H: 5.3, N: 4.91. Found - C: 67.05, H: 5.25, N: 4.72.

Preparation of Sodium 2-(4-(N-salicyloyl)aminophenyl)propionate (Sodium Salt of Carrier B)

A solution of 53.05 g (0.186 mol) of 2-(4-(N-salicyloyl)aminophenyl)propionic acid and 300 ml of ethanol was treated with 7.59 g (0.190 mol) of NaOH dissolved in 22 ml of water. The reaction mixture was stirred for 30 min at 25°C and for 30 min at 0°C. The resulting pale yellow solid was isolated by filtration to give 52.61 g of sodium 2-(4-(N-salicyloyl)aminophenyl)propionate.

Properties. Solubility: 200 mg/ml clear solution, pH=6.85. Anal. calculated for - C: 60.45, H: 5.45, N: 3.92, Na: 6.43. Found - C: 60.84, H: 5.87, N: 3.85, Na: 6.43. Melting point 236-238°C.

Preparation of the Sodium Salt of Carrier C

A 2L round bottom flask equipped with a magnetic stirrer and a reflux condenser was charged with a suspension of 3-(4-aminophenyl)propionic acid (15.0 g, 0.084 moles, 1.0 equiv.) in dichloromethane (250 ml). Chlorotrimethylsilane (18.19 g, 0.856 moles, 2.0 equiv.) was added in one portion, and the mixture was heated to reflux for 1.5 h under argon. The reaction was allowed to cool to room temperature and was placed in an ice bath (internal temperature < 10C). The reflux condenser was replaced with an addition funnel containing triethylamine (25.41 g., 0.251 moles, 3.0 equiv.). The triethylamine was added dropwise over 15 min, and a yellow solid formed during the addition. The funnel was replaced by another addition funnel containing a solution of 2,3-dimethoxybenzoylchloride (18.31 g, 0.091 moles, 1.09 equiv.) in dichloromethane (100mL). The solution was added dropwise over 30 min. The reaction was stirred in the ice bath for another 30 min and at ambient temperature for 3 h. The dichloromethane was evaporated *in vacuo* to give a brown oil. The brown oil was cooled in an ice bath, and an ice-cold solution of saturated sodium bicarbonate (250 ml) was added. The ice bath was removed, and the reaction was stirred 1 h to afford a clear brown solution. The solution was acidified with concentrated HCl and stored at *ca* 5C for 1 hour. The mixture was extracted with dichloromethane (3x100mL), dried over sodium sulfate, the salts filtered off and the dichloromethane removed *in vacuo*. The resulting solid was recrystallized from 50% ethyl acetate/water (v/v) to afford Carrier C acid as off white needles (25.92g, 90%). Anal. Calcd. For $C_{19}H_{21}NO_5$: C 66.46; H 6.16; N 4.08. Found: C 66.14; H 6.15; N 3.98. mp = 99-102C.

12 grams of the Carrier C acid was dissolved in ethanol, 75 mL, with warming. To this solution a 8.5 M Sodium hydroxide (1.02 molar equivalents, 1.426 grams in 4.5mL water) solution was added. The mixture was stirred for 15 minutes. Approximately three quarters of the ethanol was removed *in vacuo* and n-heptane, 100mL, was added to the resulting oil causing a precipitate to form. The solids were dried *in vacuo* at 50° C. Anal. Calc'd. For $C_{19}H_{20}NO_5Na \cdot 0.067H_2O$: C, 62.25; H, 5.54; N, 3.82; Na, 6.27. Found: C, 62.37; H, 5.77; N, 3.80; Na, 5.75.

Preparation of N-(4-methylsalicyloyl)-8-aminocaprylic acid (Carrier D)

(a) Preparation of Oligo(4-methylsalicylate)

Acetic anhydride (32 mL, 34.5 g, 0.338 mol, 1.03 eq), 4-methylsalicylic acid (50 g, 0.329 mmol, 1.00 eq), and xylenes (100 mL) were added to a 1 L, four-neck flask fitted with a magnetic stir bar, a thermometer, and a condenser. The flask was placed in a sand bath and heating

5 of the cloudy white mixture begun. The reaction mixture cleared to a yellow solution around 90°C. Most of the volatile organics (xylenes and acetic acid) were distilled into the Dean-Stark trap over three hours (135-146°C). Distillation was continued for another hour (a total of 110 mL distilled), during which the pot temperature slowly rose to 204°C and the distillate slowed to a trickle. The residue was poured off while still hot into an aluminum tray. Upon cooling a brittle yellow glass
10 formed. The solid was ground to a fine powder. The oligo(4-methylsalicylate) received was used without further purification.

(b) Preparation of N-(4-methylsalicyloyl)-8-aminocaprylic acid

A 7M solution of potassium carbonate (45 mL, 43.2 g, 0.313 mol, 0.95 eq), 8-aminocaprylic acid (41.8 g, 262 mol, 798 eq), and water (20 mL) were added to a 1 L round bottom
15 flask equipped with a magnetic stir bar, condenser, and an addition funnel. The white cloudy mixture was treated with a solution of oligo(4-methylsalicylate) (44.7 g, 0.329mmol 1.0 eq) and dioxane (250 mL), added over thirty minutes. The reaction mixture was heated to 90°C for 3 hours (at which time the reaction was determined to have finished, by HPLC). The clear orange reaction mixture was cooled to 30°C and acidified to pH = 2 with 50% aqueous sulfuric acid (64 g). The
20 resulting solid was isolated by filtration. The white solid was recrystallized from 1170 mL of 50% ethanol-water. The solid was recovered by filtration and was dried over 18 hours in a 50°C vacuum oven. The N-(4-methylsalicyloyl)-8-aminocaprylic acid was isolated as a white solid (30.88g, 52%); mp = 113-114°C; ¹H NMR (DMSO-d₆) δ 12.80 (s, 1H), 12.00 (s, 1H), 8.73 (bt, 1H), 7.72 (d, 1H), 6.70 (s, 1H), 6.69 (d, 1H), 3.26 (q, 2H), 2.26 (s, 3H), 2.19 (t, 2H), 1.49 (m, 4H), 1.29
25 (m, 6H). Anal. Calcd for C₁₆H₂₃NO₄: C 65.51; H 7.90; N 4.77. Found: C 65.48; H 7.84; N 4.69.

Example 2 - Pulmonary Porcine Insulin Delivery

Materials and Procedures

Materials

- 30 • Sprague Dawley female rats 225-300g (Charles River, Raleigh, NC)
- Fiber optic laryngoscope (Custom Manufactured, EPA, Research Triangle Park, NC)
- Heating blanket thermostatically control via rectal probe (Harvard Apparatus, Cambridge, HA)
- Siliconized Eppendorf tubes
- 35 • Helix Medical Silicone Tubing
- Spray instillator (Penn Century, Philadelphia, PA)

- 5 • Ketamine (100 mg/ml) Lot number 440350 (Fort Dodge Laboratories Inc. Fort Dodge, Iowa)
- Xylazine (100 mg/ml) Lot number 116ZZ01 (Vedco Inc. St. Joseph, MO)
- Acepromazine (10 mg/ml) Lot number 3941077 (Fort Dodge Laboratories Inc. Fort Dodge, Iowa)
- 10 • Heparin 1000 units/ml Lot number 104067 (Elkins-Sinn, Inc. Cherry Hill, NJ)
- 0.9% Sodium Chloride Injection USP sterile solution Lot number PS059113 (Baxter Healthcare Corporation, Deerfield, IL)
- Lyophilized porcine insulin 25.9 IU/mg (Emisphere Technologies)
- Carrier B Na-2-(4-(N-salicyloyl)amino phenyl)propionate (Emisphere Technologies)

15

Procedures

1. Solutions

- A cocktail of anesthetic solution containing Xylazine (100 mg/ml) Ketamine (100 mg/ml): Acepromazine (10 mg/ml) in the proportion of 1:3:1.
- 20 • 0.9% Sodium Chloride Injection USP containing Heparin 20 units/ml
- Insulin was dissolved in pH=3 distilled water titrated with 0.01 N HCl. When solution was clear, pH 7 distilled water was added. Then, pH was brought to 7.5 with 0.01 NaOH.
- Carrier B was dissolved in distilled water pH 7.4, sonicated for 2 minutes and its pH adjusted to 7.4 using NaOH 0.1N.

25

2. Animals

Each rat was weighed and identified using an indelible marker and anesthetized by intraperitoneal injection of ketamine: Xylazine: Acepromazine cocktail, at 80 mg/kg, 10 mg/kg and 2.0 mg/kg, respectively and returned to its cage. The rat was transferred to the surgical bench and placed on a heating blanket thermostatically controlled via rectal probe, once deep anesthesia was

30 achieved.

The right jugular vein of each rat was catheterized using silicone tubing and the patency of the catheter was confirmed by slowly flushing the cannula with 200 µl heparin saline and then withdrawing 100 µl of blood. The cannula was then cleared with 200 µl of heparinized saline solution.

35

An endotracheal tube was inserted using a fiber optic laryngoscope. The syringe (Hamilton syringe) of the instillator was attached to the endotracheal tube and used to instill

5 solution (100 µl) in the airways. The endotracheal tube was removed following administration, and the breathing rate was monitored throughout the remainder of the study.

Blood samples (500 µl) were withdrawn at 0, 10, 30, 60, 90, 120 and 180 min and the cannula flushed with approximately 200 µl of heparinized saline solution. The blood was collected in 1 ml tuberculin syringes (containing 100 µl heparin 20 µ/ml) placed directly into
10 microcentrifuge tubes.

Blood samples were immediately centrifuged at 10 000 rpm for 4 min. Aliquots of approximately 30 µl were transferred to labeled siliconized 1 ml Eppendorf tubes and kept on ice until completion of the study for subsequent glucose determination. The remaining samples were frozen at -70°C for insulin determination (Ultra-sensitive RIA).

15 Analyses

1. Glucose Determination

Determination of fasting plasma glucose levels was performed using the Ektachem DT Slide (GLU) method. The analysis is based on the enzyme-catalyzed reaction of glucose with
20 molecular oxygen, followed by a second reaction that produces a highly-colored red dye. The intensity of the color is proportional to the amount of glucose in the sample.

2. Pharmacokinetic analysis

Average insulin plasma concentrations over time were subjected to standard
25 compartmental and non-compartmental pharmacokinetic analysis. Normally, compartmental and non-compartmental analyses are performed simultaneously to demonstrate the validity of the compartmental model.

a. Compartmental analysis

30 Average insulin concentrations (C_{INS}) versus-time profiles upon spray-intratracheal (IT) administration of porcine insulin were fitted to a one-compartment body model, first order absorption using the least-squares nonlinear regression method (PKanalyst, Micromath, Salt Lake City, UT). Average concentrations of insulin were fitted to the following equation:

$$C_{PINS} = A (e^{-k_e t} + e^{-k_a t}) \quad (\text{Eq.1})$$

35 where $A = D k_a / V_d (k_a - k_e)$ is the concentration of drug in the body at time zero, k_a is the first order absorption rate constant, k_e is the first order elimination rate constant.

5 The area under the lung concentration-time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule. Interpolation to infinity was performed by dividing the last C_{ins} measured by the first-order rate constant of the terminal phase. The total area under the curve was estimated by the summation of these two compounds.

10 The elimination half-life was calculated from $0.693/k_e$ where k_e is the slope of the terminal phase of the log of the concentration-time profile.

b. Non-compartmental analysis

15 The non-compartment analysis for insulin plasma concentrations over time was performed using standard techniques. The time to peak C_{Pins} (T_{max}) and peak C_{Pins} (C_{max}) were determined from the non-fitted average plasma concentrations (C_{Pins}) versus-time profiles for the treatments. AUC was calculated by the trapezoidal rule. MRT was not calculated due to a lack of data following intravenous administration from which to calculate pharmacokinetic parameters.

3. Pharmacodynamic analysis

20 The percentage minimum plasma glucose concentration (%MPGC) and the time T to attain each %MPGC (T%MPGC) were determined from the average plasma glucose levels versus-time profiles for the treatments.

25 The area above the curve effect (AAEC) was calculated as follows:

$$AAEC = \text{Total area} - AUC_E \quad (\text{Eq.2})$$

where AUC_E represents the area under the curve effect calculated using the trapezoidal rule.

 The percentage total reduction in plasma glucose from 0-t (%TRPG 0-t) was determined using the following equation:

$$30 \quad \%TRPG \ 0-t = 100 (AAEC/\text{total area}) \quad (\text{Eq.3})$$

4. Statistical analysis

35 Differences in AUCs between insulin doses were tested for significance using Sheffe's multiple comparison test assuming $\alpha < 0.05$. Differences in $AAEC_{0-3}$ (glucose plasma levels) between insulin alone and combined with Carrier B was tested for significance using Turkey's test and analysis of variance assuming $\alpha < 0.05$.

Example 2a

(i) The plasma insulin and plasma glucose-time profiles following lung spray-intratracheal [IT] instillation of insulin 0.05 mg/kg combined with Carrier B 5 mg/kg are shown in Figures 1 and 2, respectively.

(ii) and (iii) Figures 3 and 4 and Table 1, below show the plasma glucose-time profiles and Pharmacodynamic parameters following lung spray-IT instillation of Carrier B 16 mg/kg combined with insulin 0.05 and 0.01 mg/kg respectively. A significant difference ($p < 0.05$) in the $AACE_{0-3}$ in the presence and absence of carrier for both doses of insulin was observed. The $\%TRGP_{0-3}$ increased from 10.5 ± 1.5 to 36 ± 9 % and from 47 ± 10 to 65.7 ± 5 % for the 0.01 and 0.05 doses of insulin (combined with 16 mg/kg of Carrier B), respectively. No significant difference in $\%TRGP_{0-3}$ was observed between insulin alone at 0.05 mg/kg and combined with carrier B 5 mg/kg. This dose-effect relationship found for Carrier B is most likely due to its effect on the pharmacokinetics of insulin (increased bioavailability).

Table 1

| Porcine Insulin dose (mg/kg) and Carrier B (mg/kg) | | | | | |
|---|---------------------------|--|---------------------------|---|--|
| | Insulin alone 0.01 | Insulin 0.01 & Carrier B 16 mg/kg | Insulin alone 0.05 | Insulin 0.05 & Carrier B (5 mg/kg) | Insulin 0.05 & Carrier B (16 mg/kg) |
| %MPGC | 70.02 | 46.4 | 35.7 | 38.6 | 14.9 |
| T%MPGC (min) | 180 | 90 | 120 | 90 | 60 |
| %TRPG_{0-3hr} | 10.5 ± 1.5 | 36 ± 9 | 47 ± 10 | 46 ± 8 | 65.7 ± 5 |
| AACE_{0-t} (mcU/min/ml) | 1892 ± 989 | 6395 ± 1609 | 8497 ± 1716 | 8218 ± 1430 | 11834 ± 872 |

These data suggest the potential of Carrier B to increase significantly the bioavailability of insulin and its effect on the glucose levels.

(iv) Average insulin plasma concentrations following spray-IT instillation as a function of insulin dose is shown in Fig. 5. The pharmacokinetic parameters resulting from the

5 compartmental and non-compartmental analysis are listed in Table 2, below. Non-linear curve fitting of the average plasma levels indicated that the fall in plasma concentrations was best described by a monoexponential decline with first order absorption in all cases. The evaluation of goodness of fit was done by comparing the respective model selection criteria (MSC). This modified Akaike information criterion allows the comparison of the appropriateness of a model.
10 MSC values range from 1.3 to 3.8 indicating the appropriateness of the model.

Average peak plasma concentration achieved in these studies were 57.5 $\mu\text{U/ml}$ ($T_{\max}=10$ min), 18.8 $\mu\text{U/ml}$ ($T_{\max}=20$ min), 71.3 $\mu\text{U/ml}$ ($T_{\max}=10$ min), 48.95 $\mu\text{U/ml}$ ($T_{\max}=10.0$ min), 213.1 $\mu\text{U/ml}$ ($T_{\max}=10$), 354 $\mu\text{U/ml}$ ($T_{\max}=20$), and 902 $\mu\text{U/ml}$ ($T_{\max}=20$) for the 0.001 mg/kg (0.026 U/kg), 0.005 (0.13 U/kg), 0.01 (0.26 U/kg), 0.05 (1.3 U/kg), 0.1 (2.6 U/kg), 0.5 and
15 1 mg/kg of porcine insulin, respectively and are in close agreement with those obtained after curve fitting.

The log plasma concentration-time plots as a function of insulin dose (Fig. 5) showed a decline in plasma concentrations with a defined half-life significantly longer (from 27.8 to 142.4, min see Table 2) than that previously reported following intravenous administration of
20 0.1 u/kg bovine insulin to rabbits ($t_{1/2}=30$ min). This observation suggests the presence of a "flip-flop" case, which occurs when absorption rather than elimination dictates the decline in plasma concentrations. This increase in absorption half-life does not imply however, that absorption is the rate-limiting step. Rather, this event may be the result of the slow "dissolution" or transformation of insulin from the hexameric to the monomeric form as the concentration of insulin
25 in the instilled volume increases.

The area under the curve to infinity ($\text{AUC}_{0-\infty}$) calculated using the trapezoidal rule was 2930, 1334, 5050, 5260, 15374, 67676, and 200230 $\mu\text{U min/ml}$ for the 0.001 mg/kg (0.026 U/kg), 0.005 (0.13 U/kg), 0.01 (0.26 U/kg), 0.05 (1.3 U/kg), 0.1 (2.6 U/kg), 0.5 and 1 mg/kg of porcine insulin, respectively.

30 Multiple comparison among doses using Sheffe's test revealed no significant difference ($p>0.05$) in the AUC values. This lack of significance is likely to be due to the high intrasubject variability (see, SD for AUC in Table 2). The relative bioavailability of the insulin used can be calculated assuming that the extent of absorption of porcine insulin following pulmonary administration and human insulin following subcutaneous (SC) are equivalent. Based
35 on this assumption, the relative bioavailability of porcine insulin is 12.46%.

Table 2

| Porcine Insulin dose (mg/kg) | | | | | | | |
|---|-------|-------|-------|--------|--------|-------|---------|
| | 0.001 | 0.005 | 0.01 | 0.05 | 0.1 | 0.5 | 1.0 |
| Compartmental analysis | | | | | | | |
| C _{max} (μ U/ml) | 57.2 | 16.46 | 70.5 | 48.3 | 188.08 | 381.8 | 891.3 |
| T _{max} (min) | 10.8 | 12.4 | 11.9 | 14 | 13.7 | 31.3 | 13.32 |
| AUC _{0-∞} (μ U.min/ml) | 2930 | 1334 | 5049 | 5260 | 15374 | 67676 | 2000230 |
| k _e (min ⁻¹) | 0.23 | 0.23 | 0.25 | 0.23 | 0.23 | 0.1 | 0.3 |
| t _{1/2} (elim)(min) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 7.8 | 2.1 |
| k _a (min ⁻¹) | 0.025 | 0.016 | 0.018 | 0.0105 | 0.012 | 0.007 | 0.005 |
| t _{1/2} (abs)(min) | 27.8 | 42.6 | 38.0 | 65.9 | 59.9 | 100.8 | 142.4 |
| Non-compartmental analysis | | | | | | | |
| C _{max} (ng/ml) | 57.5 | 18.8 | 71.3 | 48.95 | 213.1 | 354.1 | 902.8 |
| t _{max} (h) | 10 | 20 | 10 | 10 | 10 | 60 | 20 |
| AUC _{0-∞} (μ U.min/ml) | 1695 | 1176 | 4995 | 5288 | 17907 | 65077 | 199818 |

The plasma glucose-time profiles and PD data for the various doses of insulin instilled are shown in Figure 6 and Table 3, below. The results show that an increase in dose (from 0.01 to 1 mg/kg) produced a significant decrease in the percentage minimum plasma glucose (%MPGC) from 70.2 to 13.1, without changing significantly the time T to reach this minimum (T%MPGC). The total reduction in plasma glucose from 0 to 3 hr (%TRPG_{0-3hr}) (See Table 3) increase significantly from 10.5 to 73.7% indicating that a greater bioavailability was achieved with an increase in the extent of absorption as the insulin dose was increased.

Table 3

| Porcine Insulin dose (mg/kg) | | | | | |
|-------------------------------------|-------------|-------------|-------------|-----------------|-------------|
| | 0.01 | 0.05 | 0.1 | 0.5 | 1.0 |
| %MPGC | 70.2 | 35.69 | 35.3 | 30.6 | 13.1 |
| T%MPGC | 180 | 120 | 120 | 60 | 120 |
| %TRPG _{0-3hr} | 10.5 ± 1.5 | 47.2 ± 9.5 | 50.5 ± 12.7 | 54.5 ± 6.5 | 73.7 ± 3.1 |
| AACE _{0-t} (μU.min/ml) | 1892 ± 989 | 8497 ± 1716 | 9102 ± 2295 | 10205 ± 1173 | 13266 ± 570 |

The intersubject variability in the glucose response (CV <30%) was smaller than the insulin response. Thus, there is a dampening of the insulin variability as the hormone's appearance in the circulation is translated into its biological activity.

Example 2b

A composition of 0.05 mg/kg of porcine insulin and 16 mg/kg of Carrier B was administered to rats by lung spray-IT instillation. A composition of 0.05 mg/kg porcine insulin alone was also administered to rats by lung spray-IT instillation. Results are illustrated in Figure 7 and in Tables 4 and 5.

Table 4

| Time (min) | With Carrier B | | Without Carrier B | |
|---------------|--|----------|--|----------|
| | Average Insulin Concentration (mcU/mL \pm stdev) | AUC | Average Insulin Concentration (mcU/mL \pm stdev) | AUC |
| 0 | 0 | 10347.35 | 0 | 4300.025 |
| 10 | 157.67 \pm 32.86 | | 87.28 \pm 34.34 | |
| 30 | 101.36 \pm 35.14 | | 43.64 \pm 17.96 | |
| 60 | 70.48 \pm 30.31 | | 21.50 \pm 3.80 | |
| 90 | 44.17 \pm 8.97 | | 14.13 \pm 4.49 | |
| 120 | 37.28 \pm 15.47 | | 10.89 \pm 3.31 | |
| 180 | 11.04 \pm 3.21 | | 11.38 \pm 9.54 | |

- 5 **Table 5:** PK parameters following lung spray-IT instillation of insulin combined with carrier B and to the lungs of rats.

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| | | | |
|--|------------|-------------|--------------|
| | | | |
| Porcine Insulin dose (mg/kg) | 0.01 | 0.05 | 0.05 |
| Carrier B dose (mg/kg) | 16 | 5 | 16 |
| Compartmental Analysis (average data) | | | |
| C _{max} (μU/ml) | 31 | 54 | 147 |
| T _{max} (min) | 11 | 15 | 13 |
| AUC _{0-∞} (μU min/ml) | 1805 | 6189 | 10666 |
| k _e (min ⁻¹) | 0.21 | 0.21 | 0.21 |
| t _{1/2} (elim)(min) | 2.8 | 3.3 | 3.3 |
| k _a (min ⁻¹) | 0.022 | 0.010 | 0.017 |
| t _{1/2} (abs)(min) | 31 | 69 | 40 |
| Non-compartmental analysis | | | |
| C _{max} (μU/ml) | 31.41 | 60.7 | 158 |
| t _{max} (min) | 10 | 30 | 10 |
| AUC _{0-∞} (μU min/ml) | 1705 ± 126 | 5256 ± 3271 | 10347 ± 3089 |

Comparative Example 2c

25

Compositions of porcine insulin in escalating doses as shown in Table 6, below were administered to rats by lung spray-IT instillation. Results are illustrated in Figure 8 and Tables 6 and 7.

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Table 6: PK parameters following spray-IT instillation of escalating doses of porcine insulin to the lungs of rats.

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| Porcine Insulin dose (mg/kg) | | | | | |
|--|----------|-------------|-------------|---------------|----------------|
| | 0.01 | 0.05 | 0.1 | 0.5 | 1.0 |
| Compartmental Analysis (average data) | | | | | |
| C _{max} (μU/ml) | 6.5 | 74 | 141 | 566 | 1634 |
| T _{max} (min) | 11.3 | 11.6 | 12.4 | 16.3 | 25 |
| AUC _{0-∞} (μU min/ml) | 440 | 3989 | 9111 | 83404 | 223595 |
| k _e (min ⁻¹) | 0.25 | 0.21 | 0.20 | 0.21 | 0.21 |
| t _{1/2} (elim)(min) | 2.8 | 3.3 | 3.3 | 3.3 | 3.3 |
| k _a (min ⁻¹) | 0.018 | 0.024 | 0.02 | 0.008 | 0.009 |
| t _{1/2} (abs)(min) | 39 | 28 | 35 | 90 | 76 |
| Non-compartmental analysis | | | | | |
| C _{max} (μU/ml) | 6 | 87 | 152 | 581 | 1926 |
| t _{max} (min) | 10 | 10 | 10 | 30 | 30 |
| AUC _{0-∞} (μU min/ml) | 503 ± 93 | 4300 ± 1114 | 9517 ± 3255 | 60344 ± 16406 | 178177 ± 17152 |

Table 7

| Insulin Plasma Concentrations (mcU/ml) | | | | | | | | | | |
|---|---------------|---------------|---------------|--------------|-----------------|---------------------------|-------------|------------|------------|----------|
| Insulin Dose (mg/kg) | | | | | | Standard Deviation | | | | |
| Time (min) | 0.01 | 0.05 | 0.1 | 0.5 | 1 | 0.01 | 0.05 | 0.1 | 0.5 | 1 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 5.1 | 87.3 | 152 | 443.7 | 1198 | 2.76 | 34 | 36.8 | 214 | 220.6 |
| 30 | 4.12 | 43.6 | 91.2 | 580.5 | 1925 | 1.28 | 18 | 20.5 | 220 | 465.6 |
| 60 | 3.87 | 21.5 | 50.6 | 553.3 | 1056 | 2.76 | 3.9 | 21.6 | 219 | 304 |
| 90 | 2.27 | 14.13 | 48.7 | 275.1 | 681.7 | 0.87 | 4.5 | 38.4 | 136 | 46.9 |
| 120 | 1.87 | 10.9 | 31.1 | 211 | 919.3 | 1.57 | 3.3 | 20.9 | 88 | 323.6 |
| 180 | 1.43 | 11.4 | 19.3 | 161 | 618.3 | 0.37 | 9.5 | 5.38 | 97.6 | 216.3 |
| AUC | 490.75 | 4300.9 | 9517.5 | 60345 | 178143.5 | | | | | |

Example 2d

A composition of 0.05 mg/kg of porcine insulin at 5 mg/kg of Carrier B was administered to rats by lung spray-IT instillation. A composition of 0.05 mg/kg porcine insulin alone was also administered by lung spray-IT instillation. Insulin plasma levels are illustrated in Figure 9 and in Tables 5 and 8.

Table 8

| Time (min) | With Carrier B | | Without Carrier B | |
|---------------|--|----------|--|----------|
| | Average Insulin Concentration (mcU/mL \pm stdev) | AUC | Average Insulin Concentration (mcU/mL \pm stdev) | AUC |
| 0 | 0 | 5256.463 | 0 | 4300.025 |
| 10 | 40.49 \pm 23.30263 | | 87.28 \pm 34.34 | |
| 30 | 60.70 \pm 33 | | 43.64 \pm 17.96 | |
| 60 | 41.22 \pm 27.10568 | | 21.50 \pm 3.80 | |
| 90 | 21.08 \pm 14.46818 | | 14.13 \pm 4.49 | |
| 120 | 15.25 \pm 15.31622 | | 10.89 \pm 3.31 | |
| 180 | 19.22 \pm 16.82827 | | 11.38 \pm 9.54 | |

Example 2e

A composition of 0.01 mg/kg of porcine insulin and 16 mg/kg of Carrier B was administered to rats by lung spray-IT instillation. A composition of 0.01 mg/kg porcine insulin alone was also administered to rats by lung spray-IT instillation. Insulin plasma levels are illustrated in Figure 10 and in Tables 5 and 9.

5

Table 9

10

15

| Time (min) | With Carrier B | | Without Carrier B | |
|---------------|--|----------|--|-------|
| | Average Insulin Concentration (mcU/mL \pm stdev) | AUC | Average Insulin Concentration (mcU/mL \pm stdev) | AUC |
| 0 | 0 | 1705.418 | 0 | 502.5 |
| 10 | 31.41 \pm 8.31 | | 5.89 \pm 2.76 | |
| 30 | 16.41 \pm 4.29 | | 4.12 \pm 1.28 | |
| 60 | 10.98 \pm 6.09 | | 3.87 \pm 2.76 | |
| 90 | 4.87 \pm 1.19 | | 2.27 \pm 0.87 | |
| 120 | 5.03 \pm 1.74 | | 1.87 \pm 1.57 | |
| 180 | 4.07 \pm 2.73 | | 1.43 \pm 0.37 | |

Example 3 - Pulmonary Porcine Insulin Delivery

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Example 3a

25

A pulmonary delivery dosing composition of 0.1 mg/kg of porcine insulin and 7.5 mg/kg of Carrier B sodium salt in water was prepared. Control dosing compositions of 0.1 mg/kg insulin alone and of 7.5 mg/kg Carrier B sodium salt alone were also prepared. A 0.3 ml/kg dose of the pulmonary dosing composition at pH 7.3-7.6 was administered to five normal, non-fasting rats by the following procedure. 1½ Popper and Sons gavage needles were inserted about a few centimeters down the animals' throats. The tip of the needle was manipulated towards the animals' ventral sides where the needle could fall into a pocket and then with further manipulation the trachea. Once the needle was in the trachea, the dosing solution was delivered through the needle.

30

Periodic blood samples were drawn via the tail artery, and blood glucose levels were measured using Ektachem DT slides (Johnson & Johnson Clinical Diagnostics, Inc., Rochester N.Y.).

Results are illustrated in Figure 11 for 0.1 mg insulin with 7.5 mg/kg Carrier B sodium salt (■), 0.1 mg/kg insulin alone (), and 7.5 mg/kg Carrier B sodium salt alone (▲).

5

Example 3b

The procedure of Example 3a was repeated substituting dosing compositions of 0.5 mg/kg of porcine insulin and 7.5 mg/kg of Carrier B sodium salt, in water for the dosing composition at pH 6.6-6.9. Dosing compositions of 0.5 mg/kg insulin alone and of 7.5 mg/kg Carrier B sodium salt alone were also prepared.

Results are illustrated in Figure 12, where (■) represents 0.5 mg/kg insulin with 7.5 mg/kg Carrier B sodium salt; (●) represents 0.5 mg/kg insulin alone; and (▲) represents 7.5 mg/kg Carrier B sodium salt alone.

Example 4 - Pulmonary Insulin Delivery

Carrier compound B, carrier compound C sodium salt, and carrier compound D in Table 10 were tested as follows. Each rat was weighed and identified using an indelible marker and anesthetized by intramuscular injection of thiorazine (3 mg/kg) and ketamine (44 mg/kg).

An endotracheal tube (spray instillator from Penn Century of Philadelphia, PA) with a syringe (Hamilton syringe) attached to the endotracheal tube was inserted using a fiber optic laryngoscope. The syringe (Hamilton syringe) of the instillator was used to instill 0.4 ml/kg of a solution containing insulin (0.03 mg/kg) and a carrier compound (16 mg/kg) into the lower portions of the airway. The endotracheal tube was removed following administration, and the breathing rate was monitored throughout the remainder of the study.

Blood samples were withdrawn at 0, 5, 15, 30, 60, and 120 minutes via the tail artery and assayed with a DSL Insulin Kit #10-1600 following the procedure outlined in the kit. The serum insulin levels are illustrated in Figure 13 and the C_{max} are illustrated in Table 10 below.

Table 10

| Carrier | C_{max} (μ U/ml) |
|---------------|-------------------------|
| B | 44.6 ± 10.0 |
| C sodium salt | 22.5 ± 4.0 |
| D | 58.8 ± 12.0 |
| None | 19.4 ± 4.3 |

5 The above mentioned patents, applications, test methods, and publications are hereby incorporated by reference in their entirety.

 Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed description. All such obvious variations are within the full intended scope of the appended claim.

WHAT IS CLAIMED IS:

1 1. A method for administering a biologically-active agent to an animal in need
2 of said agent, said method comprising administering via the pulmonary route to said animal a
3 composition comprising (A) an active agent and (B) a carrier comprising an acylated amino acid,
4 a sulfonated amino acid, a polyamino acid which includes an acylated amino acid, a polyamino
5 acid which include a sulfonated amino acid, or any combination of any of the foregoing.

1 2. A method as defined in claim 1 wherein said active agent is selected from the
2 group consisting of a biologically active agent, a chemically active agent, or a combination thereof.

1 3. A method as defined in claim 2, wherein said biologically active agent
2 comprises at least one peptide, mucopolysaccharides, carbohydrate, or lipid.

1 4. A method as defined in claim 3, wherein said biologically active agent is
2 selected from the group consisting of growth hormones, human growth hormones (hGH),
3 recombinant human growth hormones (rhGH), bovine growth hormone, porcine growth hormones,
4 growth hormone-releasing hormones, interferons, α -interferon, β -interferon, γ -interferon,
5 interleukin-1, interleukin-II, insulin, insulin-like growth factor (IGF), IGF-1, heparin,
6 unfractionated heparin, heparinoids, dermatans, chondroitins, low molecular weight heparin, very
7 low molecular weight heparin, ultra low molecular weight heparin, calcitonin, salmon calcitonin,
8 eel calcitonin, human calcitonin, erythropoietin (EPO), atrial natriuretic factor, antigens, monoclonal
9 antibodies, somatostatin, protease inhibitors, adrenocorticotropin, gonadotropin releasing hormone,
10 oxytocin, leutinizing-hormone-releasing-hormone, follicle stimulating hormone,
11 glucocerebrosidase, thrombopoietin, filgrastim, prostaglandins, cyclosporin, vasopressin, sodium
12 chromoglycate, disodium chromoglycate, vancomycin, desferrioxamine (DFO), parathyroid
13 hormone (PTH), fragments of PTH, antimicrobials, anti-fungal agents, analogs, fragments,
14 mimetics and polyethylene glycol (PEG)-modified derivatives of these compounds; and any
15 combination thereof.

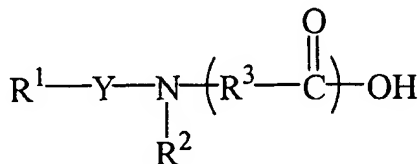
1 5. A method as defined in claim 3, wherein said biologically active agent is

selected from the group consisting of human growth hormones (hGH), recombinant human growth hormones (rhGH), bovine growth hormone, growth hormone-releasing hormones, interferons, α -interferon, β -interferon, γ -interferon, interleukin-1, interleukin-II, insulin, insulin-like growth factor (IGF), IGF-1, heparin, unfractionated heparin, heparinoids, low molecular weight heparin, very low molecular weight heparin, ultra low molecular weight heparin, calcitonin, salmon calcitonin, eel calcitonin, human calcitonin, erythropoietin (EPO), atrial natriuretic factor, antigens, monoclonal antibodies, somatostatin, adrenocorticotropin, gonadotropin releasing hormone, oxytocin, leutinizing-hormone-releasing-hormone, follicle stimulating hormone, glucocerebrosidase, thrombopoietin, filgrastim, prostaglandins, cyclosporin, vasopressin, sodium chromoglycate, disodium chromoglycate, vancomycin, desferrioxamine (DFO), parathyroid hormone (PTH), fragments of PTH, antimicrobials, anti-fungal agents, analogs, fragments, mimetics and polyethylene glycol (PEG)-modified derivatives of these compounds; and any combination thereof.

6. A method as defined in claim 3, wherein said biologically active agent comprises an interferon, interleukin-II, insulin, insulin-like growth factor (IGF), IGF-1, heparin, low molecular weight heparin, low molecular weight heparin, calcitonin, oxytocin, vasopressin, vancomycin, desferrioxamine, parathyroid hormone, and combinations thereof.

7. A method as defined in claim 2, wherein said biologically active agent selected from the group consisting of insulin, insulin-like growth factor (IGF), IGF-1, or combinations thereof.

8. The method of claim 1, wherein the carrier comprises a compound having the formula



wherein

R^1 is $\text{C}_1\text{--C}_7$ alkyl, $\text{C}_3\text{--C}_{10}$ cycloalkyl, cycloalkenyl, aryl, thienyl, phenyl, naphthyl, pyrrolo, or pyridyl;

R^1 is optionally substituted with one or more $\text{C}_1\text{--C}_7$ alkyl, $\text{C}_2\text{--C}_7$ alkenyl, $\text{C}_2\text{--C}_7$

alkynyl, C₆-C₁₀ cycloalkyl, phenyl, phenoxy, F, Cl, Br, -OH, -SO₂, -SO₃H, -NO₂, -SH, -PO₃H, oxazolo, isoxazolo, alkoxy having the formula -OR⁶, -COOR⁷, -N(R⁵)₂, -N⁺(R⁵)₃X⁻, or any combination thereof;

Y is $\text{---}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{---}$ or -SO₂-;

X is halogen, hydroxide, sulfate, tetrafluoroborate, or phosphate;

R² is hydrogen, C₁-C₄ alkyl, C₂-C₄ alkenyl, or -(CH₂)_n-COOH, where n is 1 to 10;

R³ is C₁-C₂₄ alkyl, C₂-C₂₄ alkenyl, C₂-C₂₄ alkyne, C₃-C₁₀ cycloalkyl, C₃-C₁₀ cycloalkenyl, phenyl, naphthyl, (C₁-C₁₀ alkyl) phenyl, (C₂-C₁₀ alkenyl) phenyl, (C₁-C₁₀ alkyl) naphthyl, (C₂-C₁₀ alkenyl) naphthyl, phenyl (C₁-C₁₀ alkyl), phenyl (C₂-C₁₀ alkenyl), naphthyl (C₁-C₁₀ alkyl) or naphthyl (C₂-C₁₀ alkenyl);

R³ being optionally substituted with C₁-C₄ alkyl, C₂-C₄ alkenyl, C₁-C₄ alkoxy, -OH, -SH, halogen, -NH₂, -CO₂R⁴, C₃-C₁₀ cycloalkyl, C₃-C₁₀ cycloalkenyl, heterocyclic having 3-10 ring atoms wherein the hetero atom is one or more of N, O, S or any combination thereof, aryl, (C₁-C₁₀ alk)aryl, ar(C₁-C₁₀ alkyl), or any combination thereof;

R⁴ is hydrogen, C₁-C₄ alkyl, or C₂-C₄ alkenyl;

R⁵ is hydrogen or C₁-C₁₀ alkyl;

R⁶ is C₁-C₁₀ alkyl, alkenyl, alkynyl, aryl or cycloalkyl; and

R⁷ is hydrogen, C₁-C₁₀ alkyl, alkenyl, alkynyl, aryl or cycloalkyl.

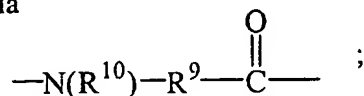
9. The method of claim 8, wherein the active agent is selected from the group consisting of insulin, insulin-like growth factor (IGF), IGF-1, or combinations thereof.

10. The method of claim 1, wherein the carrier comprises a compound having the formula Ar-Y-(R⁸)_n-OH, wherein

Ar is a substituted or unsubstituted phenyl or naphthyl, preferably Ar is substituted or unsubstituted 2-OH-phenyl;

Y is $\text{---}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{---}$ or -SO₂-;

R⁸ has the formula

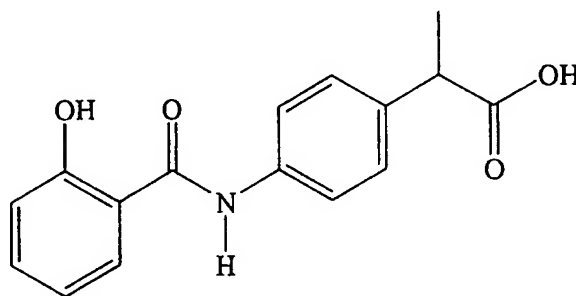


R⁹ is C₁ to C₂₄ alkyl, C₁ to C₂₄ alkenyl, phenyl, naphthyl, (C₁ to C₁₀ alkyl) phenyl, (C₁-C₁₀ alkenyl) phenyl, (C₁ to C₁₀ alkyl) naphthyl, (C₁ to C₁₀ alkenyl) naphthyl, phenyl (C₁ to C₁₀

alkyl), phenyl (C_1 to C_{10} alkenyl), naphthyl (C_1 to C_{10} alkyl) and naphthyl (C_1 to C_{10} alkenyl);
 R^9 is optionally substituted with C_1 to C_4 , alkyl, C_1 to C_4 alkenyl, C_1 to C_4 alkoxy,
 $-OH$, $-SH$, $-CO_2R^{11}$, cycloalkyl, cycloalkenyl, heterocyclic alkyl, alkaryl, heteroaryl, heteroalkaryl,
 or any combination thereof;
 R^9 is optionally interrupted by oxygen, nitrogen, sulfur or any combination thereof;
 R^{10} is hydrogen, C_1 to C_4 alkyl or C_1 to C_4 alkenyl; and
 R^{11} is hydrogen, C_1 to C_4 alkyl or C_1 to C_4 alkenyl.

11. The method of claim 10, wherein the active agent is selected from the group consisting of insulin, insulin-like growth factor (IGF), IGF-1, or combinations thereof.

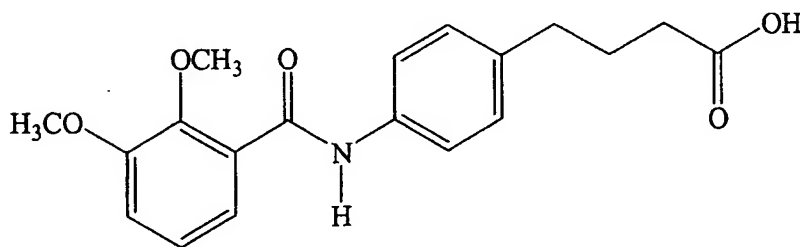
12. The method of claim 1, wherein the carrier comprises a compound having the formula



and salts thereof.

13. The method of claim 12, wherein the active agent is selected from the group consisting of insulin, insulin-like growth factor (IGF), IGF-1, or combinations thereof.

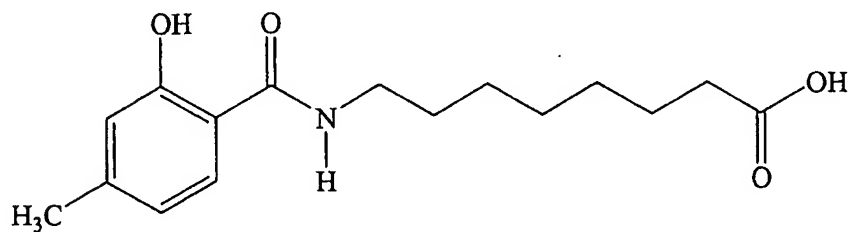
14. The method of claim 1, wherein the carrier comprises a compound having the formula



or a salt thereof.

1 15. The method of claim 14, wherein the active agent is selected from the group
2 consisting of insulin, insulin-like growth factor (IGF), IGF-1, or combinations thereof.

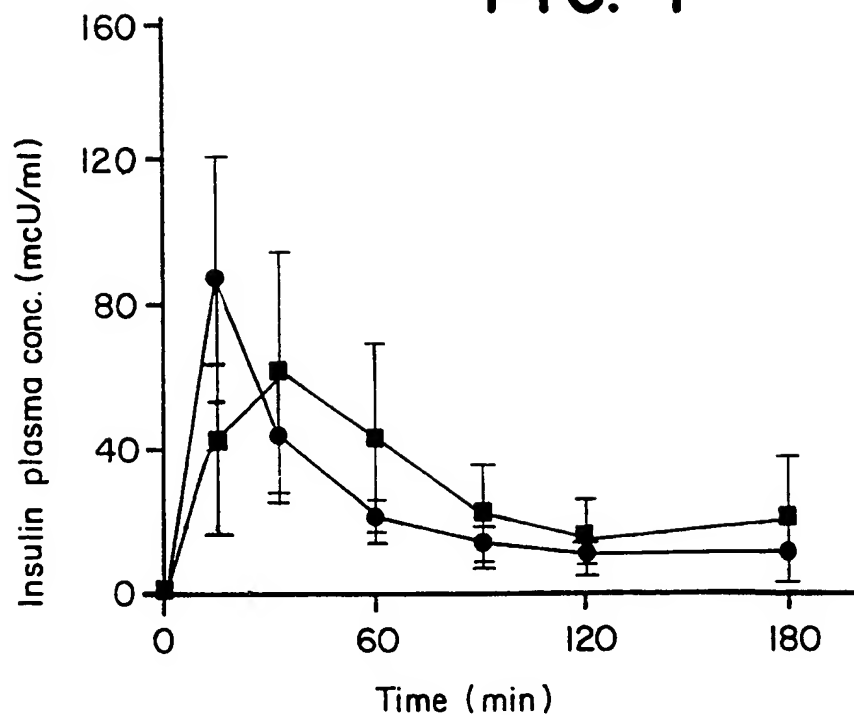
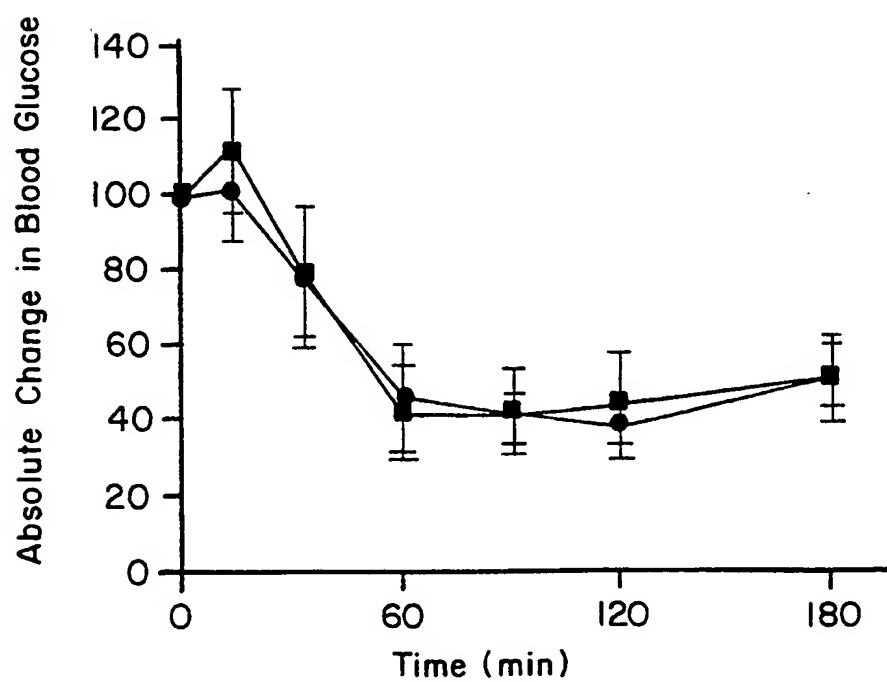
1 16. The method of claim 1, wherein the carrier comprises a compound having
2 the formula



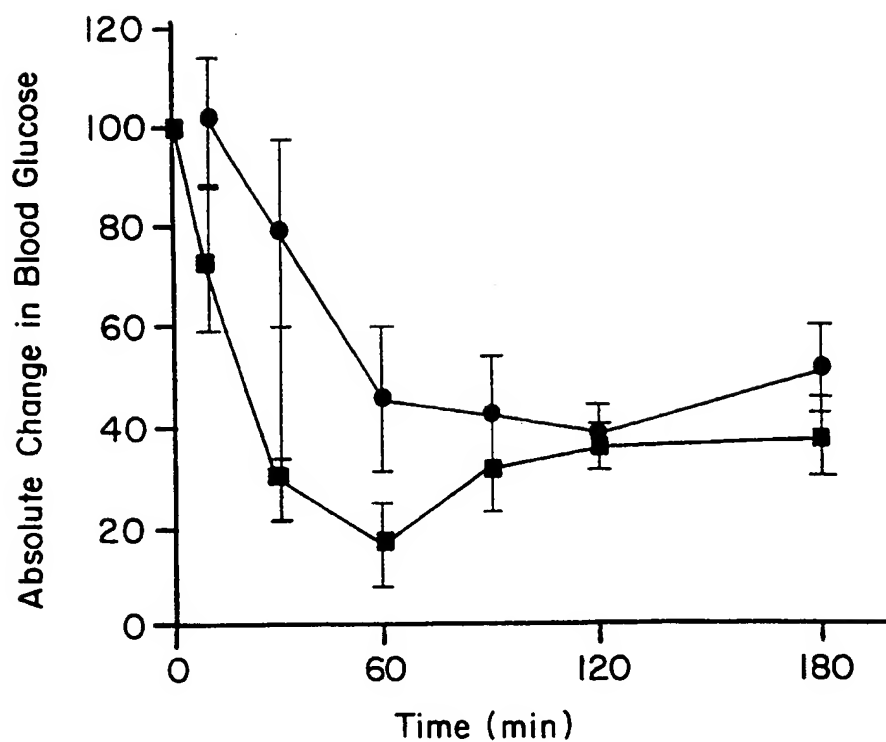
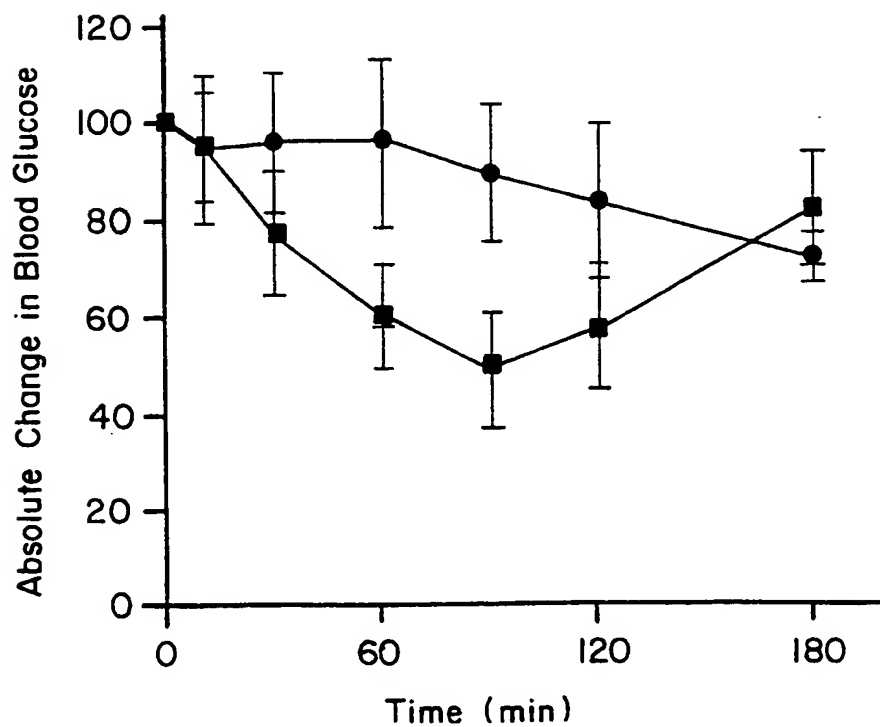
8 or a salt thereof.

1 17. The method of claim 16, wherein the active agent is selected from the group
2 consisting of insulin, insulin-like growth factor (IGF), IGF-1, or combinations thereof.

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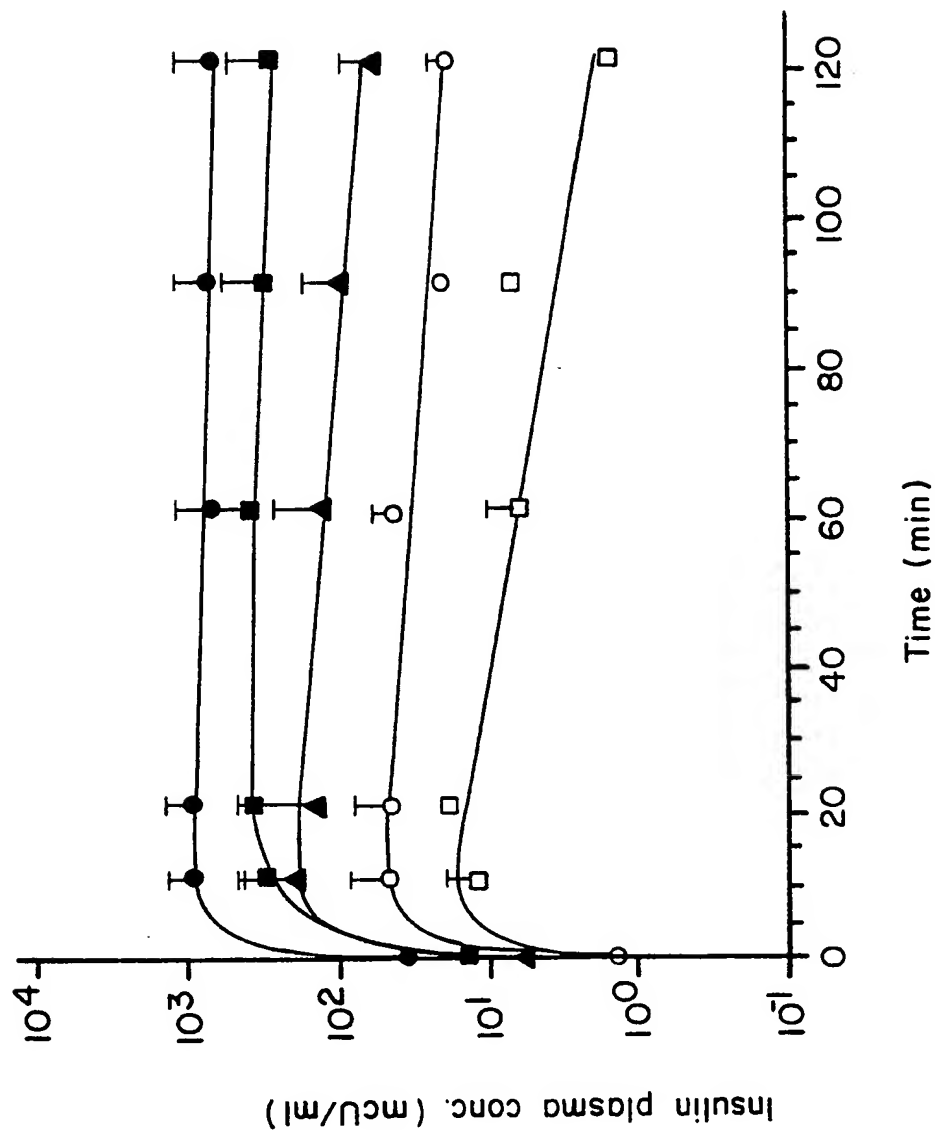
FIG. 1**FIG. 2**

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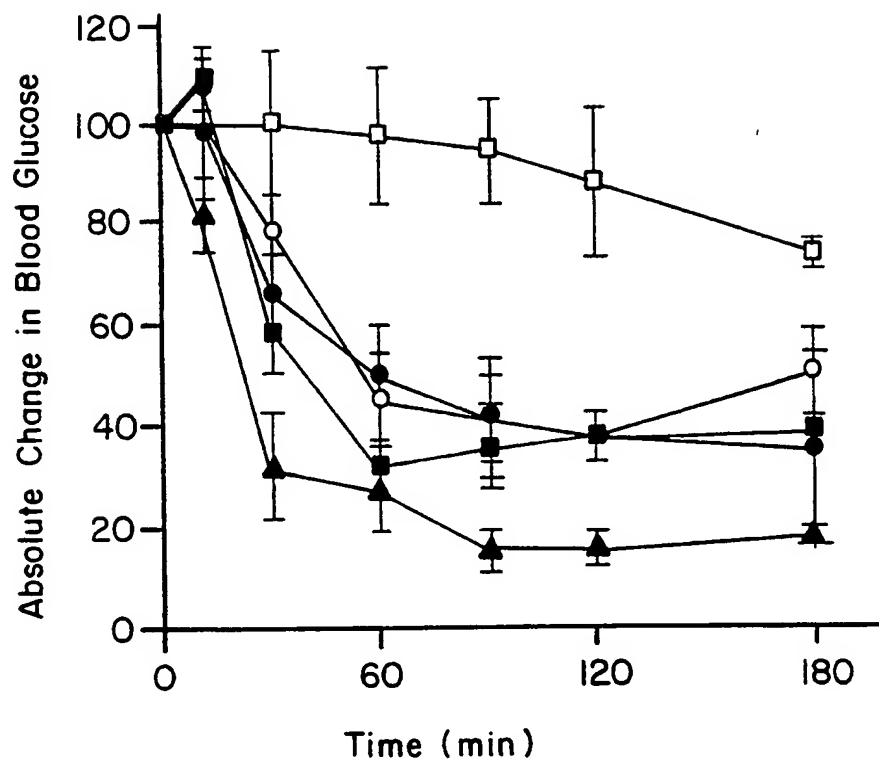
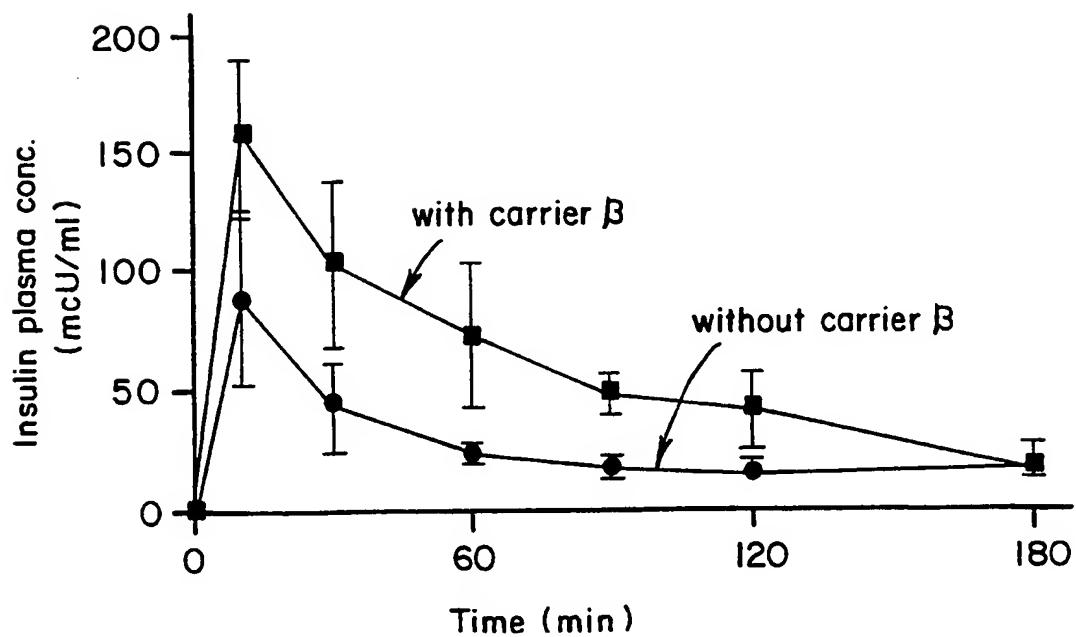
FIG. 3**FIG. 4**

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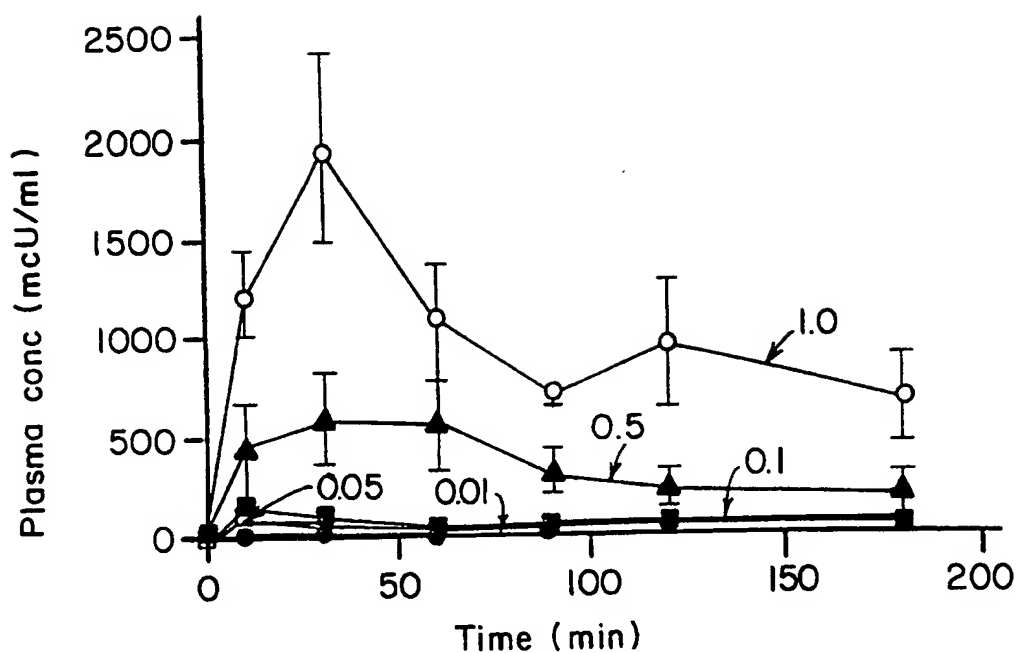
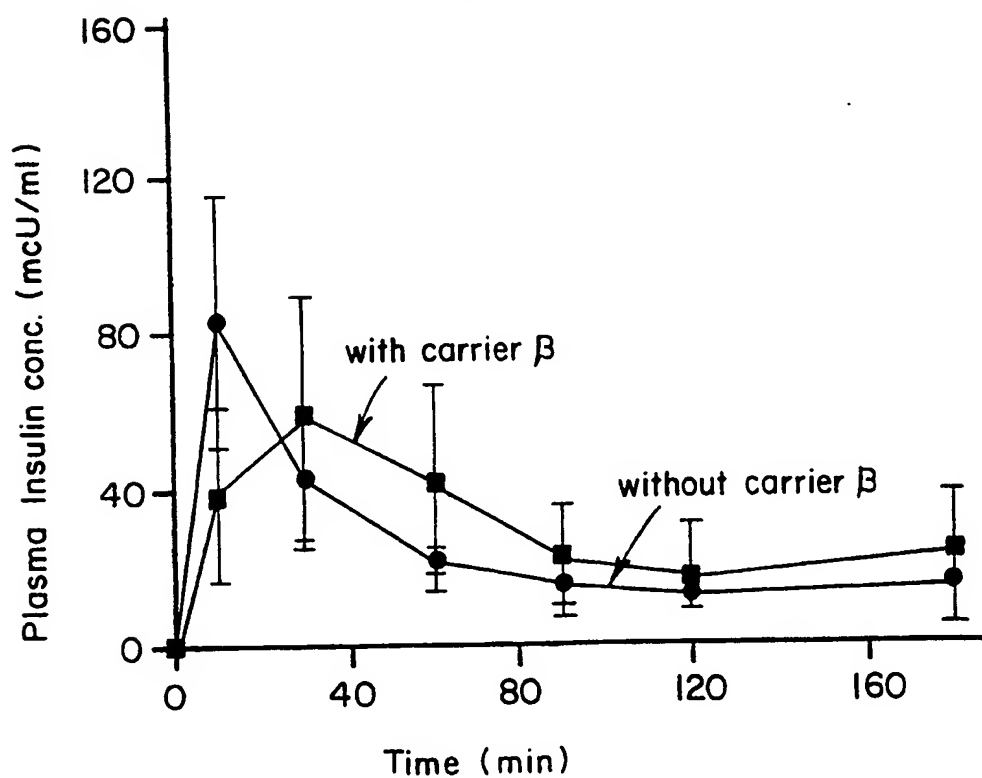
FIG. 5



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FIG. 6**FIG. 7**

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FIG. 8**FIG. 9**

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FIG. 10

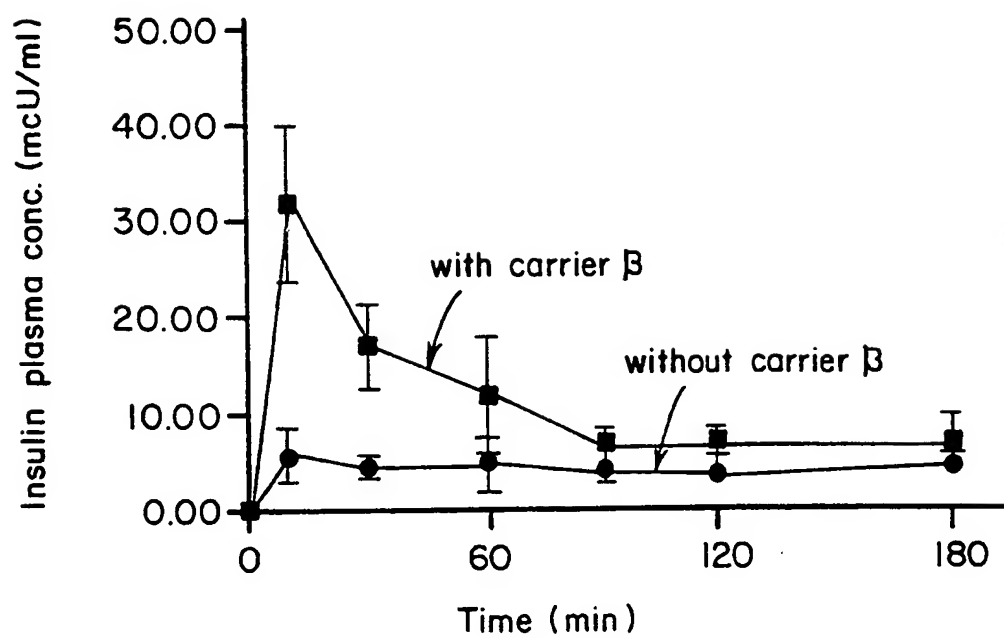
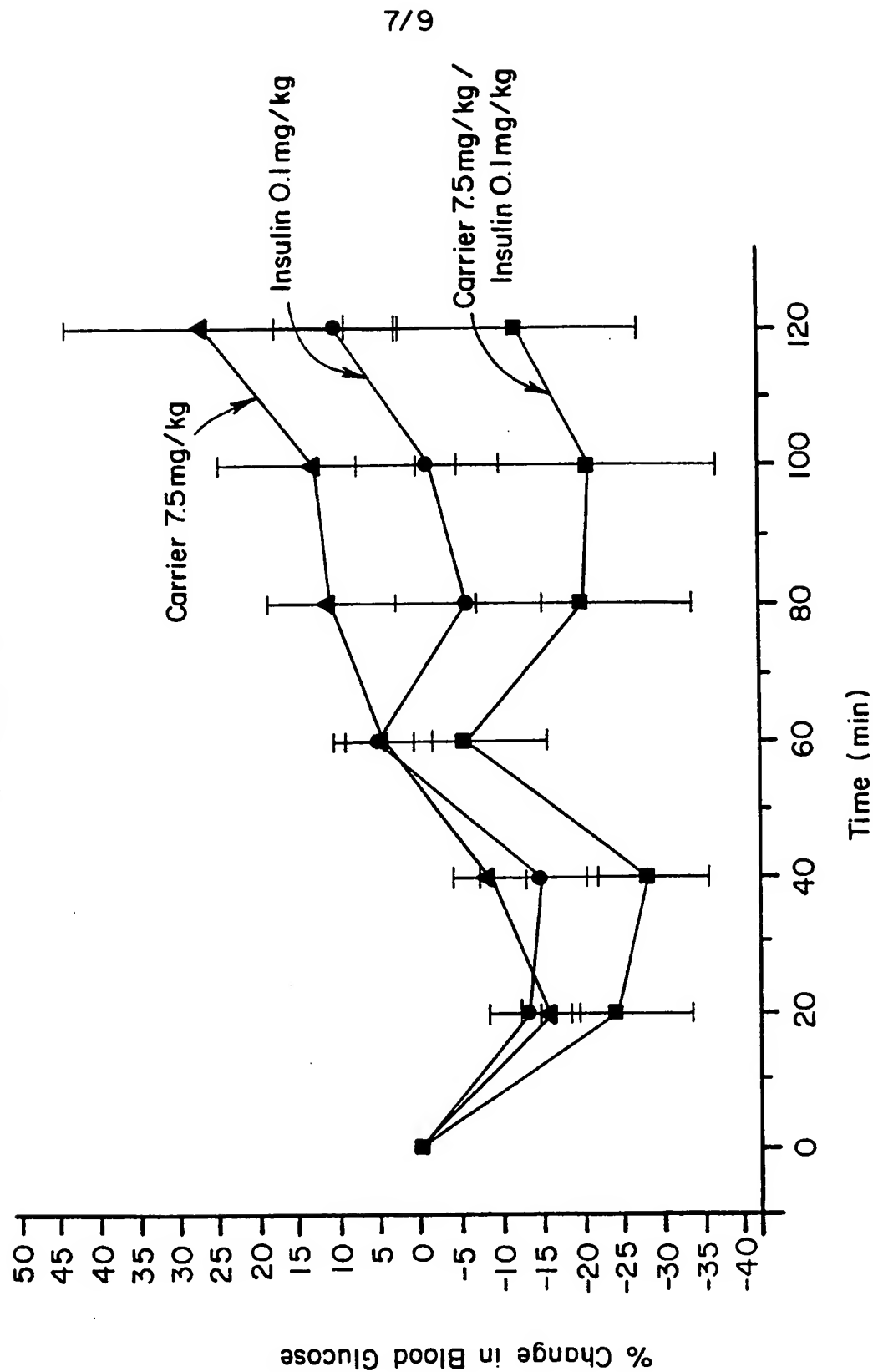
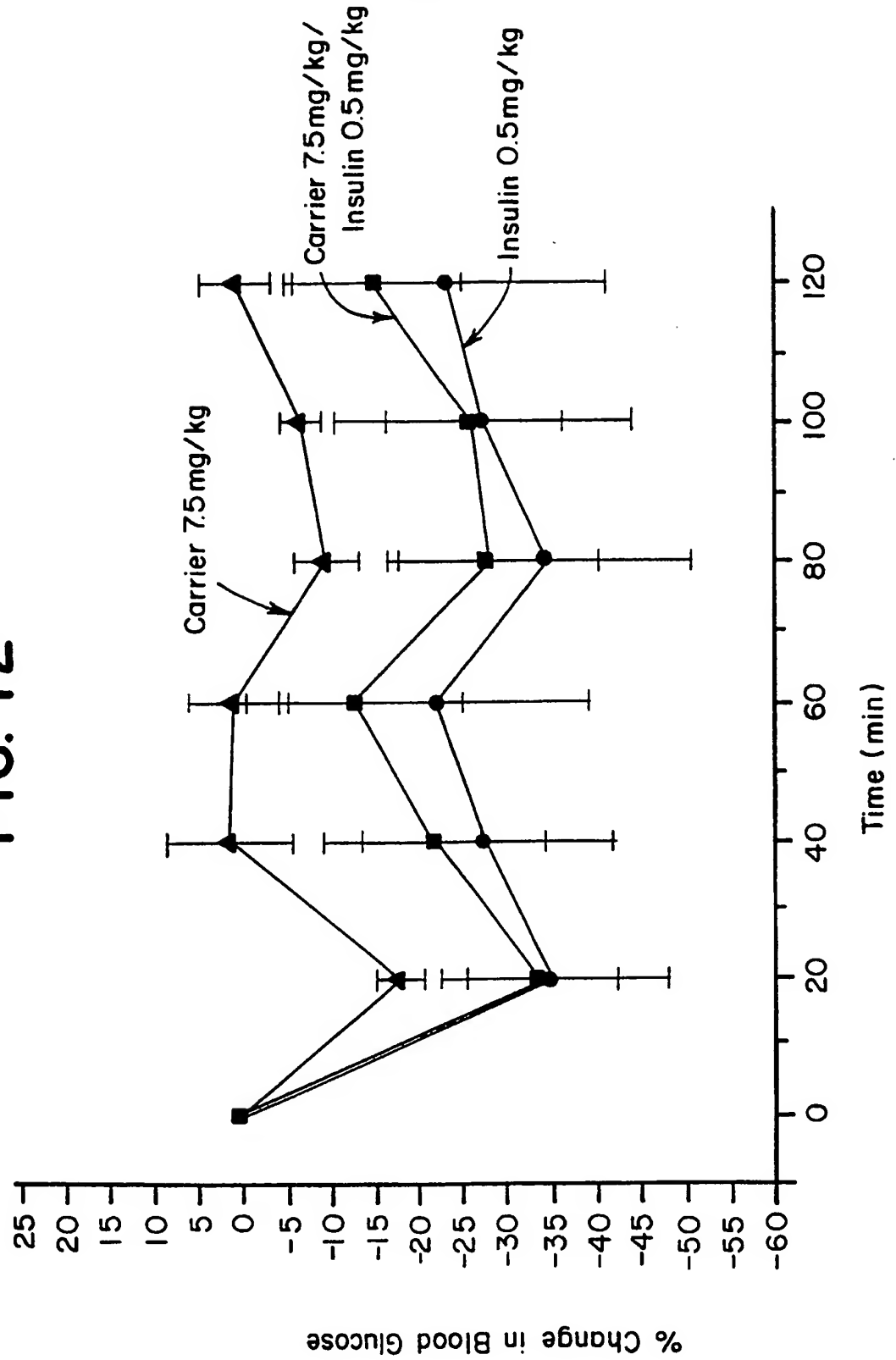


FIG. 11



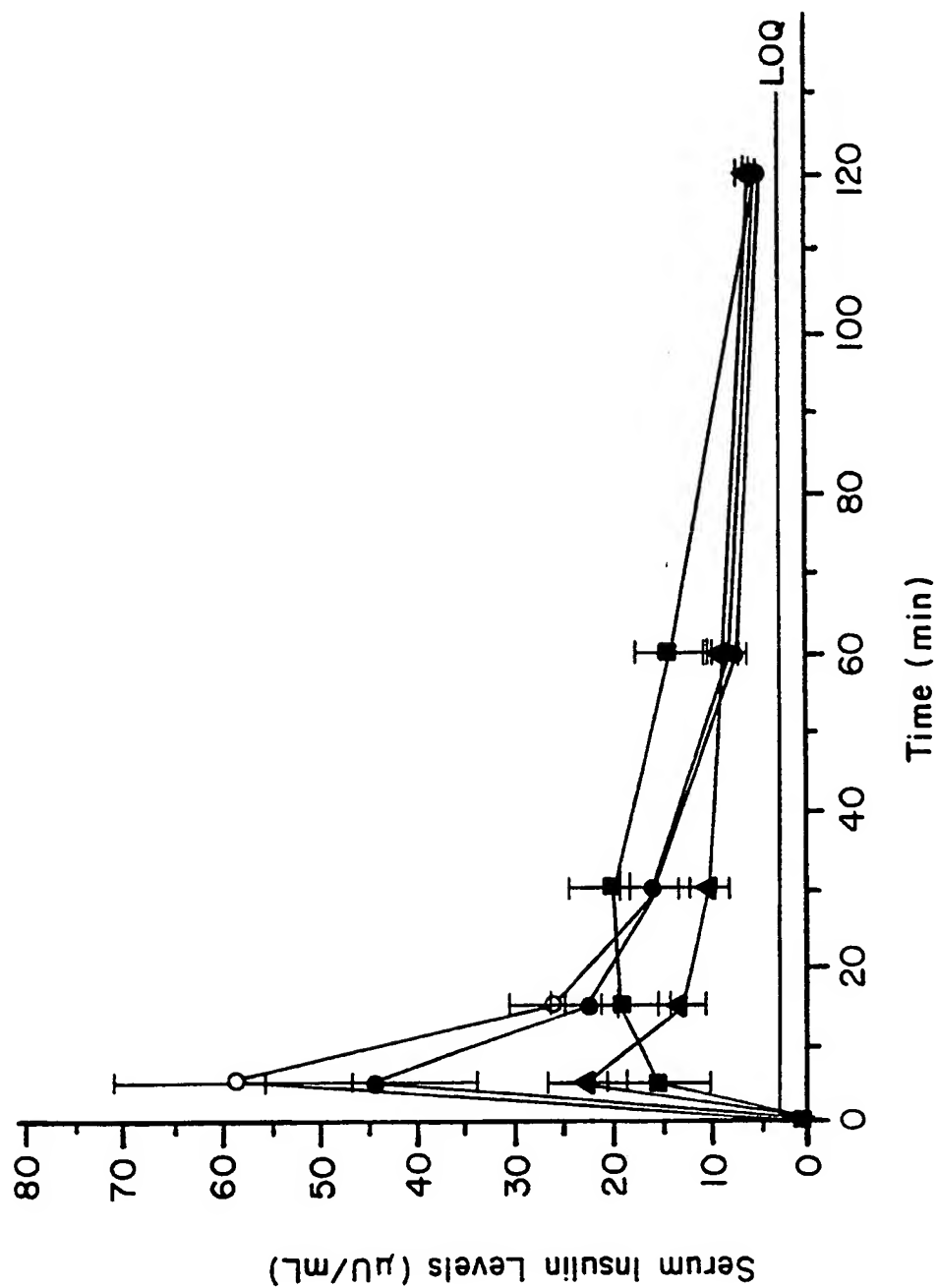
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FIG. 12



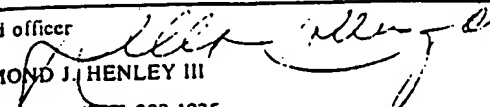
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FIG. 13



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16957

| A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : A61K 38/00, 31/725, 31/195 US CL : 514/2, 11, 12, 21, 56, 563 According to International Patent Classification (IPC) or to both national classification and IPC | | |
|--|--|--|
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/2, 11, 12, 21, 56, 563 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | US 5,650,386 A (LEONE-BAY et al.) 22 July 1997. | 1-17 |
| A,P | US 5,863,944 A (LEONE-BAY et al.) 26 January 1999. | 1-17 |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | |
| * Special categories of cited documents: | *T | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
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| *P* document published prior to the international filing date but later than the priority date claimed | | |
| Date of the actual completion of the international search 12 OCTOBER 1999 | Date of mailing of the international search report 03-NOV 1999 | |
| Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230 | Authorized officer  RAYMOND J. HENLEY III Telephone No. (703) 308-1235 | |